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STIMULATORY EFFECT OF IL-1β ON CATECHOLAMINE SECRETION FROM CULTURED BOVINE ADRENAL MEDULLARY CELLS

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Summary We investigated the effect of recombinant human interleukin-1β (IL-1β) on catecholamine secretion from cultured bovine adrenal medullary cells. Treatment of cultured cells with IL-1β (10 ng/ml) for 24 hr caused an increase in accumulation of catecholamines in the cultured medium. The accumulation of catecholamines stimulated by IL-1β was observed in time (4 - 48 hr)- and concentration (3 - 30 ng/ml)-dependent manners. The stimulatory effect of IL-1β (10 ng/ml) was completely inhibited by recombinant human IL-1 receptor antagonist (1 μg/ml). IL-1β had little effect on [^{3}H]norepinephrine uptake to cultured cells. These results suggest that IL-1β stimulates catecholamine secretion through activation of IL-1 receptors in adrenal medullary cells.

Interleukin-1 (IL-1) that stimulates a broad spectrum of immune and inflammatory responses was first identified as a product of lipopolysaccharide (LPS)-stimulated macrophages(1). Recent studies have proposed that IL-1 may play a role in the modulation of neuroendocrine and neural functions of the brains in addition to their roles as immune mediators (2,3). In fact, IL-1 stimulates the secretion of hypothalamic cortictropin-

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Abbreviations used; IL-1, interleukin-1; IL-1 ra, IL-1 receptor antagonist; KRP, Krebs-Ringer phosphate; LPS, lipopolysaccharide.

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releasing factor (4) and adrenocorticotropic hormone (5) in rats in vivo and the release of arginine vasopressin from the superfused hypothalamo-neurohypophyseal complexes of rats (6).

Adrenal medullary cells are derived from embryonic neural crest tissue and share many physiological and pharmacological properities with postganglionic sympathetic neurons. Stimulation of acetylcholine receptors in adrenal medulla causes a Ca^{2+} influx which triggers the secretion of catecholamines (7). Recent immunochemical studies have demonstrated the existence of IL-1 in the adrenal medulla of rats, mice (8) and rat pheochromocytoma PC12 cells (9). Furthermore, injection of LPS produced an increase in level of IL-1 immunoreactivity and nicotine reduced its level in rat adrenal medulla (10), suggesting that the expression of IL-1 is induced by LPS and IL-1 can be released by cholinergic stimulaiton. However, little is known the effect of IL-1 on the functions of adrenal medullary cells.

In the present study, we examined the effect of long exposure of IL-1β on catecholamine secretion from the cells.

Materials and Methods

Bovine adrenal medullary cells were isolated (11,12), and maintained in monolayer culture (10^6 or 2 x 10^5 cells/well, Falcon 24 or 96 well) in Eagle's minimum essential medium (Eagle's MEM) containing 10% calf serum and antibiotics (11,13). Oxygenated Krebs-Ringer phosphate (KRP) buffer was used for the experiment of [^{3}H]norepinephrine uptake. It had the following composition (mM): NaCl 154, KCl 5.6, $MgSO_4$ 1.1, $CaCl_2$ 2.2, NaH_2PO_4 0.85, Na_2HPO_4 2.15, and glucose 10, adjusted to pH 7.4.

Catecholamine accumulation in cultured medium

Cultured adrenal medullary cells (10^6 or 2 x 10^5/well) were washed 3 times with 0.5 ml of Eagle's MEM and then treated with or without IL-1β (1 - 100 ng/ml) for various periods in a CO_2-incubator (CO_2 5% - air 95%). Catecholamines accumulated in cultured medium were adsorbed to aluminum hydroxide (12) and estimated by the ethylenediamine condensation method (14) using a fluorescence spectrometer (Hitachi Model 650-10S) with an excitation wave length of 420 nm and an emission of 540 nm.

[^{3}H]Norepinephrine uptake by the cells

Cultured cells (10^6/well) were treated with or without IL-1β (10 ng/ml) for 24 hr in Eagle's MEM in the CO_2-incubator. After washing with 0.5 ml of KRP buffer 4 times, cells were incubated with [^{3}H]norepinephrine (10^{-7}M, 0.5 μCi) at 37° C for 1 hr in the presence or absence of IL-1β (10 ng/ml) and imipramine (5×10^{-6}M). The cells were rapidly washed with 0.5 ml of ice-cold KRP buffer 4 times, and solubilized in 1 ml of 10% Triton X-100. The radioactivity in the cells was counted by a Beckman LS-7000 liquid scintillation counter.

Chemicals used were obtained from the following sources; recombinat human IL-1β was a kindly gift from Y. Hirai (Otsuka Pharmaceutical, Tokushima, Japan); collagenase, Nitta Zerachin, Osaka Japan; Eagle's minimum essential medium, Nissui Seiyaku, Tokyo, Japan; recombinat human IL-1 receptor antagonist (IL-1 ra) (R&D System, Mineapolis, MN, U.S.A.); 1-[7,8-^{3}H] norepinephrine, Amersham International,Buckinghamshire, England; Other chemicals are analytical grade from Nacalai Tesque, Kyoto, Japan.

Statistical analysis: Data obtained are shown as mean ± SD (standard deviation). Statistical evaluation was performed with Student's t-test or analysis of variance (ANOVA).

Results and Discussion

IL-1β (10 ng/ml) caused an increase in accumulation of catecholamines in cultured medium of bovine adrenal medullary cells in a time-dependent manner (Fig. 1). The significant effect by IL-1β was detectable after treatment for 4 hr and observed during incubation with IL-1β for 4 - 48 hr. Exposure of cells with IL-1β for 24 hr produced a concentration (1 - 30 ng/ml)-dependent increase in catecholamine accumulation (Fig. 2). The significant increase and maximal increase by IL-1β were observed at 3 ng/ml and 30 ng/ml, respectively. The concentrations (1 - 3 ng/ml) of IL-1β used in the present study may be comparable to the serum level (0.3 - 2.3 ng/ml) of IL-1β in septic patients (15) and to IL-1β concentrations (1 ng/ml) in cerebrospinal fluid of patients with bacterial meningitis (16).

The various actions of IL-1 (IL-1α and β) are reported to be mediated by specific receptors on membranes of murine T

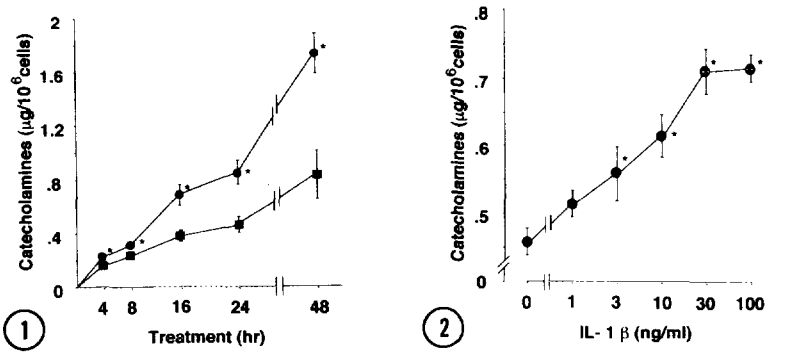

Figure 1. Time course of IL-1β-induced accumulation of catecholamines in cultured medium of adrenal medullary cells
Cultured adrenal medullary cells (10^6/0.5 ml/well) were treated with (●) or without (■) recombinant human IL-1β (10 ng/ml) for the period indicated. Catecholamines accumulated in cultured medium were measured. Data are means ± SD of 4 - 7 experiments.
*p < o.o1; compared to each control.

Figure 2. Concentration-response curve for IL-1β-stimulated catecholamine accumulation in cultured medium
Cultured cells were treated for 24 hr with various concentrations of IL-1β (0 - 100 ng/ml). Data are means ± SD of 5 - 7 experiments.
*p < 0.01; compared to IL-1β (0 ng/ml).

lymphoma cell and fibroblast cell lines (17), human and porcine fibroblasts (18). Both IL-1α and β bind to the same receptor site on human B lymphoblastoid cell line and murine T lymphoma cells (19). To test a hypothesis that IL-1β stimulates catecholamine accumulation through a specific receptor for IL-1, we used an antagonist of IL-1 receptor (IL-1ra) (20). Table 1 shows that recombinant human IL-1ra (1 μg/ml) completely inhibited the stimulatory effect of IL-1β on catecholamine accumulaiton. The result suggests that IL-1β exerts its effect via activation of IL-1 receptors in adrenal medullary cells.

Next, we studied whether the catecholamine accumulation stimulated by IL-1β is due to an increase in catecholamine secretion or a decrease in reuptake of catecholamines. After cells were treated with or without IL-1β (10 ng/ml) for 24 hr, they were incubated with [^{3}H]norepinephrine for 1 hr in the presence or absence of IL-1β (10 ng/ml) and imipramine (5 x 10^{-6}

Table 1. Effect of IL-1 receptor antagonist on catecholamine accumulation stimulated by IL-1β

	Catecholamines (μg/ 2 x 10^5 cells)
None	0.251 ± 0.029
IL-1 receptor antagonist (1 μg/ml)	0.256 ± 0.034
IL-1 β (10 ng/ml)	0.372 ± 0.043 *
IL-1 β (10 ng/ml) + IL-1 ra	0.257 ± 0.024 **

Cultured cells (2 x 10^5/0.2 ml/well, 96 well plate) were treated with or without IL-1β (10 ng/ml) and recombinant human IL-1 receptor antagonist (1 μg/ml) for 48 hr. Data are means ± SD of 4 experiments.
* $p < 0.01$; compared to none.
** $p < 0.01$; compared to IL-1β alone.

M). Table 2 shows that IL-1β had little effect on imipramine-sensitive or insensitive [^{3}H]norepinephrine uptake by the cells. These present results indicate that IL-1β increases the secretion of catecholamines from cultured adrenal medullary cells. Recently,

Table 2. Effect of IL-1β on [^{3}H]norepinephrine uptake by cultured cells

	[^{3}H] NE Uptake (10^3dpm/ 10^4cells)	
	Imipramine-sensitive	Imipramine-insensitive
None	86.3 ± 2.1	17.5 ± 0.8
IL-1 β (10 ng/ml)	91.3 ± 5.7	16.5 ± 0.7

Cultured cells (10^6/0.5 ml/well) were treated with or without IL-1β (10 ng/ml) for 24 hr. After treatment, cells were incubated at 37° C for 1 hr with or without IL-1β (10 ng/ml) and imipramine (5 x 10^{-6}M) in the presence of [^{3}H]norepinephrine (10^{-7}M, 0.5 μ Ci/well). [^{3}H]Norepinephrine (NE) taken up by the cells was measured. Imipramine-sensitive uptake was calculated by subtraction of the value obtained in the presence of imipramine from that in the absence of imipramine. Imipramine-insensitive uptake was measured in the presence of imipramine. Data are means ± SD of 4 experiments.

cytokines have been reported to modulate catecholamine release; tumor necrosis factor attenuates [^{3}H]norepinephrine release via a prolong recovery from inactivation of release in cultured rat sympathetic neurons (21) or IL-1β augments the release of norepinephrine and dopamine in rat anterior hypothalamus (22).

IL-1 has been reported to modify the signal transduction pathway. IL-1 stimulates the production of cyclic AMP in a human natural killer-like cell line (23) and of diacylglycerol in a human T cell leukemia line (24). Furthermore, IL-1 induces a sustained increase in cytosolic free Ca^{2+} in cultured rabbit osteoclasts (25). Since the second messengers described above are well known to stimulate catecholamine secretion (26), it is interesting to speculate that IL-1β changes the level of the second messenger(s) in adrenal medullary cells.

In conclusion, we have demonstrated that IL-1β enhances the secretion of catecholamines via stimulation of IL-1 receptors in cultured adrenal medullary cells.

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