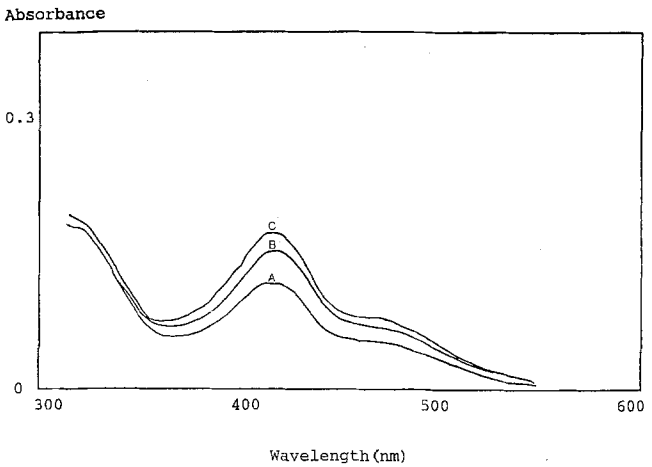

Figure 7. Exposure of catechin (final concentration = 0.5 mg/ml) to the protein fraction corresponding to peak 0.1b (50 ng protein) produced an oxidized product that absorbs maximally at 428 nm. Peak 0.1b was obtained by elution of the root exudate of lettuce seedlings with 0.1 M NaCl on DEAE Sepharose column. *A*, *B* and *C* are the absorption spectra taken at an interval of 10 min. $A = 10$, $B = 20$, $C = 30$ min.

tomato (*Lycopersicon esculentum*), alfalfa (*Medicago sativa*), rice (*Oryza sativa*) and corn (*Zea mays*) were used. All the seedlings that were screened released oxidase/s that were able to oxidize catechin.

The presence of oxidases in the tissues of plants [7] is well established, but this is the first time that it has been reported that oxidases are released from the roots of plants into the rhizosphere.

When some of the properties of these released oxidases and of other plant systemic oxidases that have been reported were compared, differences were found in the molecular weight and pH optimum [7]. However, 1-phenyl-3-(2-thiazolyl)-2-thiourea, an inhibitor that acts on the copper in the plant systemic oxidases [7], also inhibits this catechin oxidase.

Understanding of the role of this oxidase is still rudimentary, although it has been shown that it was able to reduce the growth inhibitory effects of some naturally occurring phenolics (eg. catechol and hydroquinone) through oxidation.

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ACTH response induced by interleukin-1 is mediated by CRF secretion stimulated by hypothalamic PGE

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Summary. We investigated whether hypothalamic prostaglandin E_2 (PGE_2) and corticotropin releasing factor (CRF) are responsible for the development of the adrenocorticotropic hormone (ACTH) response induced by interleukin-1α (IL-1α). The present results show that ACTH responses induced by intravenous injection of IL-1α were suppressed by systemic pretreatment with indomethacin and that intrahypothalamic injection of PGE_2 stimulates the secretion of ACTH. Furthermore, systemic pretreatment with anti-CRF antibody significantly suppressed the ACTH response induced by intrahypothalamic injection of PGE_2. These data suggest that the ACTH response induced by IL-1 is mediated by CRF secretion stimulated by hypothalamic PGE_2.
Key words. Hypothalamus; interleukin-1; prostaglandin; corticotropin releasing factor; ACTH; acute phase response.

Interleukin-1 (IL-1), which is released by circulating and reticuloendothelial monocytes in response to a variety of pathogenic stimuli such as bacterial endotoxin, induces many immune and metabolic responses for host defence [1, 2]. IL-1 activates the arachidonic acid cascade system to synthesize and release prostaglandins (PGs), which act on the hypothalamus [3, 4] as possible final mediators to induce fever [5, 6]. Furthermore, recently IL-1 was found to stimulate the secretion of adrenocorticotropic hormone (ACTH) and glucocorticoid hormone when injected systemically [7]. The involvement of PGs has also been revealed [8–10] in the ACTH response induced by IL-1, since systemic pretreatment with the inhibitor of PG synthesis, indomethacin, significantly suppressed the

secretion of ACTH after IL-1 injection. Moreover, we have recently reported that intrahypothalamic injection of prostaglandin E_2 (PGE_2) induces a significant increase in the plasma concentration of ACTH[9,10]. Therefore, it is suggested here that febrile and ACTH responses are mediated by the action on the hypothalamus of PGs induced by IL-1.

However, two hypotheses on the mechanism of ACTH response induced by IL-1 have been proposed. One is that IL-1 directly acts on the pituitary gland to stimulate the secretion of ACTH[11], and the other is that IL-1 induces ACTH response indirectly via the hypothalamic hormone, corticotropin releasing factor (CRF)[12-14]. In the present study, we investigated whether or not CRF involves the ACTH response induced by intrahypothalamic injection of PGE_2. We used the immunoneutralization method, in which ACTH secretion is suppressed; it is thought that the mechanism involved is that intravenous injection of anti-CRF antibody neutralizes CRF activity via the hypophyseal portal vein. The present results show that intrahypothalamic PGE_2 causes a significant increase in the plasma concentration of ACTH, whereas pretreatment with anti-CRF antibody suppresses the ACTH response induced by intrahypothalamic injection of PGE_2. Furthermore, the ACTH response to IL-1 is suppressed by systemic pretreatment with an inhibitor of PG synthesis, indomethacin. Therefore, it is suggested that ACTH response induced by IL-1 is mediated by CRF secretion stimulated by hypothalamic PGE_2.

Materials and methods

The animals used in this study were male albino rats of the Wistar strain, weighing 250–350 g. The rats were divided into three experimental groups: exp. 1 (n = 6) in which i.v. injection of IL-1α or saline was performed, exp. 2 (n = 11) in which rats were given indomethacin (INDO, 10 mg/kg i.v.) or control vehicle 15 min before i.v. injection of IL-1α, and exp. 3 (n = 6) in which rats were treated with anti-CRF antibody, or normal rabbit serum (NRS) as a control for anti-CRF antibody, 15 min before intrahypothalamic injection of PGE_2. In exp. 2, i.v. injection of INDO was performed at a dosage of 10 mg/kg. This dose seems to be relatively large; it was chosen because previous reports[9,10] showed the complete antipyretic action of INDO at 10 mg/kg i.v.

In the present study the group which received IL-1α after pretreatment with INDO was called the 'INDO + IL-1 group', and the group which received IL-1α after pretreatment with control vehicle was called the 'control + IL-1 group'. The groups which received intrahypothalamic injection of PGE_2 after pretreatment with anti-CRF antibody and NRS were called the 'anti-CRF + PGE_2 group' and the 'NRS + PGE_2 group', respectively. For blood sampling and intravenous injections, in all rats Silastic tubing was implanted into the superior caval vein (SCV) 2 days before blood sampling, by the transjugular cannulation technique[15]. For intrahypothalamic injections rats had been implanted previously with a stainless-steel cannula (0.8 mm, o.d.) into the preoptic and anterior hypothalamic region, at coordinate AP 1.5, L 1.5, V 8.5 mm according to the rat brain atlas[16], by standard stereotaxic techniques under general anesthesia (sodium pentobarbital 35 mg/kg, i.p.). This implantation was done at least 10 days before the start of the experiment.

Human recombinant interleukin-1α (IL-1α) was supplied by the Dainippon Pharmaceutical Co. Ltd. The recombinant IL-1α was dissolved in sterile saline at a concentration of 10 μg/ml. Prostaglandin E_2 was dissolved in saline containing 2% ethanol at a concentration of 12.5 μg/ml. Indomethacin (INDO) was dissolved in saline containing 20% ethanol and 4% sodium bicarbonate at a concentration of 8 mg/ml. We used saline containing 20% ethanol and 4% sodium bicarbonate as the control vehicle for the INDO solution. Anti-CRF antibody was purchased from Cambridge Research Biochemicals Ltd.

All injections were performed at 12.00 h. Intrahypothalamic injections were made with a stainless-steel needle (0.4 mm o.d.) attached to a polyethylene tube, and the volume infused was always 2 μl. Injection doses in each experimental group are described in the results. For measuring the plasma concentration of ACTH, about 0.5 ml of blood was withdrawn through the cannulae previously implanted into the SCV. The blood samplings were performed four times: 30 min before and 30, 90 and 180 min after the injections. To determine the plasma concentration of ACTH, a radioimmunoassay for ACTH was performed using an ACTH radioimmunoassay kit (Compagnie Oris Industrie S.A.). Data were analyzed for statistical significance using Student's t-test.

Results

Changes in the plasma concentration of ACTH after intravenous injection of IL-1α (15 μg/kg) or saline are shown in figure 1. The plasma concentration of ACTH increased significantly 30 min after i.v. injection of IL-1α. However, the elevated level of plasma ACTH returned to the pre-injection level 90 min after injections. Figure 1 also shows changes over time in the plasma concentration of ACTH after i.v. injection of IL-1α (15 μg/kg) 15 min after pretreatment with indomethacin (INDO, 10 mg/kg) or the control vehicle. The increase in the plasma concentration of ACTH 30 min after injection of IL-1α was significantly suppressed in the 'INDO + IL-1', as compared with that in the 'control + IL-1 group'. However, a significant increase in the plasma concentration of ACTH was observed 180 min after injection of IL-1α in the 'INDO + IL-1 group'.

Figure 2 shows changes in the plasma concentration of ACTH after intrahypothalamic injection of PGE_2 (25 ng) in two groups with pretreatment by i.v. administration of anti-CRF antibody (0.4 ml) or NRS (0.4 ml) 15 min before injection of PGE_2. As shown in figure 2, the

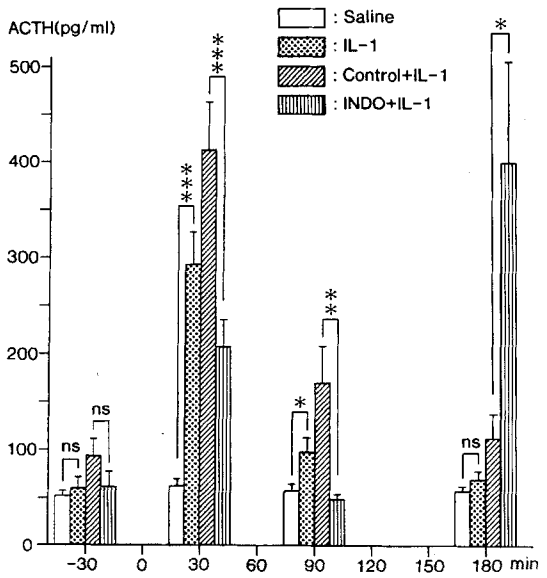

Figure 1. Changes in plasma concentration of adrenocorticotropic hormone (ACTH) after i.v. injection of interleukin-1α (IL-1, 15 μg/kg) (stippled bars) or saline (open bars), and changes in plasma concentration of ACTH after intravenous injection of IL-1 (15 μg/kg) after pretreatment with indomethacin (INDO, 10 mg/kg) (striped bars) or the control vehicle (hatched bars) 15 min before injection of IL-1. The values at each time are statistically compared between the group which received IL-1 and the group which received saline, and between the 'INDO + IL-1 group' and the 'control + IL-1 group'. ns, no significance; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

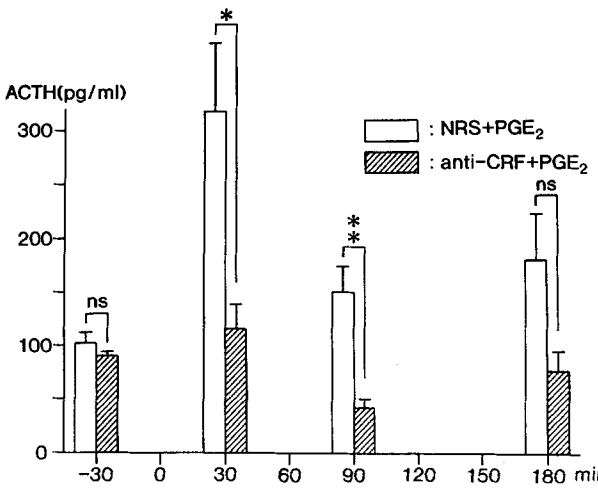

Figure 2. Changes in plasma concentration of adrenocorticotropic hormone (ACTH) after intrahypothalamic injection of prostaglandin E_2 (PGE_2, 25 ng) after pretreatment with anti-CRF antibody (hatched bars) or normal rabbit serum (NRS) (open bars) 15 min before injection of PGE_2. The values at each time are statistically compared between the 'anti-CRF + PGE_2 group' and the 'NRS + PGE_2 group'. ns, no significance; $*p < 0.05$; $**p < 0.01$.

concentration of plasma ACTH increased significantly 30 min after intrahypothalamic injection of PGE_2 in the 'NRS + PGE_2 group'. In contrast, comparing the ACTH response to the intrahypothalamic injection of PGE_2 in the 'anti-CRF + PGE_2 group' with that in the 'NRS + PGE_2 group', the increase in the plasma concentration of ACTH 30 min after intrahypothalamic injection of PGE_2 was completely suppressed by pretreatment with anti-CRF antibody.

Discussion

It has been demonstrated that IL-1 activates the pituitary-adrenocortical axis to increase plasma concentration of ACTH and glucocorticoid hormone[7]. These responses have been considered to constitute the so-called 'feed-back loop' system in which ACTH[17] and glucocorticoid hormone[18] suppresses febrile response, and glucocorticoid hormone[7] suppresses hypersecretion of IL-1. In the present results, the i.v. injection of IL-1α increased the plasma concentration of ACTH, and it is subsequently inferred that the plasma concentration of glucocorticoid hormone might increase. Furthermore, ACTH responses were suppressed by systemic pretreatment with the cyclooxygenase inhibitor, indomethacin. In addition, it was confirmed that the intrahypothalamic injection of PGE_2 increased the plasma level of ACTH concentration. This indicates that IL-1 activates the arachidonic acid cascade system to synthesize and release PGs which act on the hypothalamus to induce the ACTH response, as well as the febrile response.

However, in the present study, the plasma level of ACTH significantly increased 180 min after injection of IL-1α in the 'INDO + IL-1 group'. Although this phenomenon cannot be given a clear explanation, one possibility is that the suppression of the ACTH response by systemic pretreatment with INDO leads to a decrease in the plasma concentration of glucocorticoid hormone. Thus, the feedback inhibition of CRF by glucocorticoid hormone might be attenuated, and then a 'rebound' enhancement of the secretion of ACTH might occur. In contrast, considering that the intrahypothalamic injection of PGE_2 stimulates the secretion of ACTH, we can speculate that CRF, a hypothalamic hormone, is released in the hypophyseal portal vein. The present results clearly show the involvement of CRF in ACTH response induced by the intrahypothalamic injection of PGE_2, since the pretreatment with anti-CRF antibody significantly suppresses the ACTH secretion. In the present study PGE_2 was locally injected into the preoptic and anterior hypothalamic region. However, it still remains to be elucidated whether neurons of this region directly in response to PGE_2, release the CRF into the portal vein or stimulate the CRF containing neurons in other hypothalamic regions. In the present study, the effect of anti-CRF antibody on IL-1 induced ACTH response was not investigated. However, many previous reports have shown the complete inhibition of the ACTH response to systemically injected IL-1 by anti-CRF antibody[12-14]. Therefore the present results suggest that PGE_2, which is synthesized and released in response to IL-1, induces the ACTH response by stimulating the secretion of CRF.

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Serum thymic factor as a radioprotective agent promoting survival after X-irradiation

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Summary. Serum thymic factor (FTS, zinc-free thymulin) protected mice from death after whole-body X-irradiation. It was significantly radioprotective even when administered after irradiation, but it was more effective when administered both before and after irradiation. The protective effect appears to be due to the enhancement of hematologic recovery in the animals.
Key words. Serum thymic factor; thymulin; FTS; radioprotector; radiation protection.

Protection against ionizing radiation can be achieved by so-called chemical radioprotectors, as reviewed recently by Rojas and Denekamp[1], but clinical application of these chemicals has been limited by their toxicity. Another group of radioprotective substances are cytokines. Interleukin 1 (IL-1) increases survival of irradiated animals if it is injected 20 h before irradiation[2] or 3 h after irradiation[3]. Hematopoietic factors like granulocyte-macrophage colony-stimulating factor (GM-CSF) can enhance recovery of damaged hematopoietic tissues in post-radiation therapy, and recombinant GM-CSF has actually been used to treat victims of a radiation accident[4]. Furthermore a substance such as lipopolysaccharide (bacterial endotoxin)[5] probably exerts its radioprotective action by inducing IL-1, CSFs and other cytokines in treated animals.

In the present study, we examined the radioprotective effect of serum thymic factor (FTS) in X-irradiated mice. This factor is a thymic hormone and regulates differentiation and some functions of T lymphocytes[6,7]. Chemically, it is a nonapeptide and its amino acid sequence (pGlu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH) appears to be identical in different animal species[7]. According to Bach and co-workers, FTS requires zinc for its biological activity and the Zn(II)-FTS complex is now called thymulin[8]. Pharmacologically, therapeutic effects, e.g. on multiple sclerosis, have been proposed[9,10]. Because T-cells produce various cytokines able to stimulate macrophages and other cells of the immune system, we expected this T-cell activating hormone to exhibit a radioprotective effect by enhancing defense reactions in the body.

Materials and methods

Zinc-free FTS synthesized chemically by the conventional method was dissolved in endotoxin-free saline at 0.5 mg/ml and injected daily in 0.2-ml aliquots (i.e., 100 μg FTS per injection), subcutaneously. This dosage was determined in a previous study in which FTS was given in doses ranging from 0.1 to 1000 μg per injection to establish the optimal dose to protect lethally-irradiated mice (data not shown). X-rays were generated at 200 kVp/20 mA and filtered through 0.5 mm each of Cu and Al. Male 9-week-old mice of the C3H/HeN strain were exposed to 66–70 cGy/min to give a total dose of 567 cGy (half-lethal) or 756 cGy (lethal). The animals were observed for 30 days thereafter to measure the survival rate.