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Interleukin 1 potentiates the secretion of β-endorphin induced by secretagogues in a mouse pituitary cell line (AtT-20)

(corticotropin releasing factor/vasoactive intestinal peptide/phorbol ester/protein kinase C/forskolin)

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ABSTRACT **Previous work has shown that corticotropin releasing factor, vasoactive intestinal peptide, phorbol ester, and forskolin cause the secretion of adrenocorticotropic hormone and β-endorphin from the AtT-20 mouse pituitary cell line. Human recombinant interleukin 1α and 1β also stimulated adrenocorticotropic hormone and β-endorphin secretion from AtT-20 cells in a time- and dose-related manner. The effect appeared only after pretreatment with interleukin 1 (IL-1) for at least 18 hr and was maximum at 24 hr. After pretreatment of the cells over a period of time with IL-1, the secretion induced by corticotropin releasing factor and vasoactive intestinal peptide was increased in more than an additive manner. The enhancement of corticotropin releasing factor-induced β-endorphin release produced by IL-1 was apparent after 12 hr and reached a maximum at 24 hr. IL-1 did not affect forskolin-induced cAMP generation but enhanced the effect of forskolin on β-endorphin secretion. This suggests that IL-1 does not induce adenylate cyclase and that forskolin causes the secretion of β-endorphin by a mechanism independent of cAMP. IL-1 enhanced phorbol ester-induced β-endorphin secretion. After prolonged treatment with phorbol ester (an activator of protein kinase C), the secretion induced by phorbol ester was abolished as well as the enhancement induced by IL-1. However, prolonged treatment with phorbol ester had no effect on IL-1-induced β-endorphin secretion. These observations suggest that IL-1 enhances peptide-generated secretion of β-endorphin by inducing protein kinase C.**

Interleukin 1 (IL-1) is a polypeptide lymphokine (molecular mass of 17.5 kDa) produced by numerous cell types including macrophages, fibroblasts, and astrocytes (1). There are two distinct IL-1 genes expressing IL-1 activities, IL-1α and IL-1β. Although IL-1α and IL-1β have less than 30% amino acid homology, they interact with the same receptors (2). IL-1 has a broad spectrum of biological activities as well as an important role in modulation of the immune responses and inflammation (3). It produces fever, somnolence, anorexia, and elevation of acute-phase proteins. It has been reported that injection of IL-1 elevates plasma adrenocorticotropic hormone (ACTH) levels *in vivo* (4–7). IL-1 was found to cause ACTH release in primary cultures of anterior pituitary cells (8, 9) and in AtT-20 cells, a mouse anterior pituitary cell line (10). Other investigators could not confirm these observations (4, 7) but suggested that IL-1 acts centrally to stimulate hypothalamic corticotropin releasing factor (CRF) secretion and ACTH release from the pituitary gland.

It has been shown that CRF (11), vasoactive intestinal peptide (VIP) (12, 13), isoproterenol (14), phorbol ester (15), and forskolin (12) induce ACTH release from AtT-20 cells. To further clarify the action of IL-1 on the release of β-endorphin in AtT-20, its effect was examined over longer time periods than those reported. We found that IL-1 can induce β-endorphin secretion only after many hours of treatment. In addition, it was observed that IL-1 pretreatment potentiates the secretion of β-endorphin induced by secretagogues (CRF, VIP, phorbol ester, and forskolin) by a mechanism involving protein kinase C.

MATERIALS AND METHODS

Cell Culture. AtT-20/D16-16 mouse anterior pituitary tumor cells, which synthesize and secrete proopiomelanocortin-derived peptides (16), were obtained from S. Sabol (National Heart, Lung, and Blood Institute, Bethesda, MD) and grown in Dulbecco's modified Eagle's medium containing glucose (4.5 g/liter), 10% (vol/vol) fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The cells were maintained at 37°C in a humidified atmosphere of 10% CO_2/90% air. Cells were subcultured in 24-well Costar plates at an initial density of 5 or 10 × 10^4 cells per well and used 5–7 days later (80–90% confluency).

β-Endorphin Release Experiments. At the onset of each experiment, freshly prepared 10% fetal bovine serum/Dulbecco's modified Eagle's medium with or without IL-1 was added to the cells and the incubation was continued for the times indicated. Then AtT-20 cells were washed twice with 1 ml of 0.2% bovine serum albumin/Dulbecco's modified Eagle's medium to remove the large amounts of β-endorphin released during the long pretreatment period and incubated in identical serum-free medium in the presence or absence of IL-1 with or without other secretagogues for 60 min. The test medium was collected separately from each well and centrifuged, and the supernatant fluids were stored at −60°C until analysis. The radioimmunoassay procedure used for β-endorphin determination has been described (17). Results were expressed as ng per well per hr. Data representing β-endorphin secretion induced by secretagogues were calculated by subtracting the amount of β-endorphin released by untreated cells, with the exception of Fig. 1.

cAMP Studies. After pretreatment of the cells with or without IL-1 for 23 hr, AtT-20 cells were preincubated for 60 min in the presence of 100 μM 3-isobutyl-1-methylxanthine. Then 0.3 ml of fresh medium with 100 μM 3-isobutyl-1-methylxanthine and test substances were added to the cells. After 30 min of incubation, 30 μl of 3 M HCl was added to each well and sonicated for 10 sec. Cell suspensions were centrifuged and aliquots of supernatant fluids were vacuum-centrifuged to dryness for subsequent cAMP measurement. cAMP was determined by radioimmunoassay as described (18).

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Abbreviations: IL-1, interleukin 1; CRF, corticotropin releasing factor; ACTH, adrenocorticotropic hormone; VIP, vasoactive intestinal peptide; TPA, phorbol 12-*O*-tetradecanoate 13-acetate.

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Statistical Analysis. Experiments for β-endorphin secretion and cAMP accumulation were performed in 24-well plates. Each experimental manipulation was carried out in four to six wells. The values obtained were averaged and considered as a single experiment. Data presented are means ± SEM. Statistical comparisons were made by using a Student's test.

Materials. Dulbecco's modified Eagle's medium, fetal bovine serum, L-glutamine, penicillin–streptomycin, and phosphate-buffered saline without Ca^{2+}/Mg^{2+} were obtained from GIBCO; CRF and VIP were purchased from Peninsula Laboratories; forskolin, phorbol 12-*O*-tetradecanoate 13-acetate (TPA), and bovine serum albumin (RIA grade) were from Sigma; ^{125}I-labeled β-endorphin and ^{125}I-labeled cAMP were from New England Nuclear. IL-1α was a gift of J. Oppenheim (National Cancer Institute, Frederick, MD) and P. Lomedico (Hoffman–La Roche) and IL-1β was generously provided by Otsuka Pharmaceuticals (Rockville, MD).

RESULTS

IL-1 Induces β-Endorphin Secretion from AtT-20 Cells in a Time- and Dose-Related Manner. Human recombinant IL-1α and IL-1β stimulated β-endorphin release only after a prolonged period of incubation (Fig. 1). There was no measurable increase of β-endorphin secretion after 12 hr of pretreatment. However, after 18 hr of pretreatment, a small but significant increase in β-endorphin release was observed, reaching a maximum at 24 hr (Fig. 1). After IL-1 incubations for more than 24 hr, a significant increase was not observed. Both IL-1α and IL-1β induced β-endorphin secretion with similar potency and in a dose-dependent manner. A significant stimulation of β-endorphin release was observed at 1 pM IL-1, whereas maximum stimulation occurred at 1 nM (Fig. 2).

IL-1 Enhances CRF- and VIP-Induced β-Endorphin Secretion. The effect of IL-1 on CRF-induced β-endorphin secretion was examined. After treating AtT-20 cells with CRF alone for 1 hr, there was an expected increase in the release of β-endorphin (Fig. 3). In cells pretreated with IL-1 for 23 hr, CRF induced more than an additive secretion of β-endorphin compared to that produced by neuropeptide or lymphokine when incubated separately (Fig. 3). The enhancement of CRF-induced opioid peptide release was apparent after 12 hr of IL-1α pretreatment, reaching a maximum at 24 hr. Similar results were obtained with IL-1β. The effect of IL-1 was also examined on the ability of VIP to release β-endorphin. There

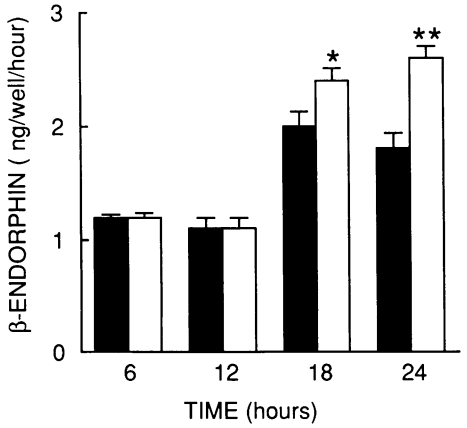

FIG. 1. IL-1 induces the secretion of β-endorphin after a period of time. AtT-20 cells were incubated for various time periods in the absence (solid bars) or in the presence (open bars) of 1 nM IL-1α. The cells were washed 1 hr prior to the end of each time period and then incubated with serum-free medium with IL-1α or without interleukin, and β-endorphin release was determined in medium collected after 60 min of incubation. The data (mean ± SEM) are representative of six experiments with similar results. $*P < 0.05$; $**P < 0.01$ (vs. control).

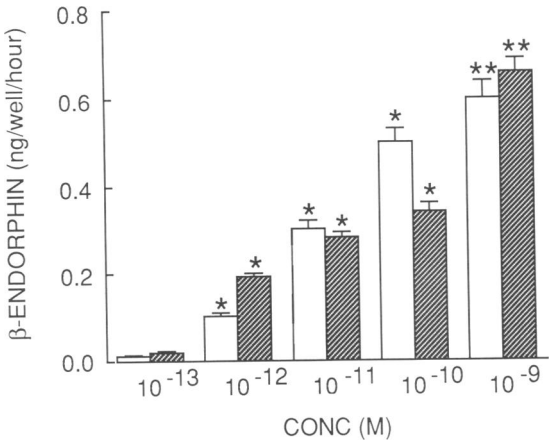

FIG. 2. Dose–response data for IL-1-induced β-endorphin secretion. AtT-20 cells were pretreated with IL-1α (open bars) and IL-1β (hatched bars) for 23 hr and then washed, and serum-free medium with interleukin was added. After a 1-hr incubation, medium from each well was collected and β-endorphin released was assayed. The secretion for different concentrations of IL-1α and IL-1β is shown after subtracting the amount of β-endorphin released by untreated cells. The results are the mean ± SEM from one of four similar experiments. $*P < 0.05$; $**P < 0.01$ (vs. control).

was about a 70% enhancement of VIP-induced β-endorphin secretion caused by lymphokine (data not shown).

IL-1 Increases Forskolin-Induced β-Endorphin Secretion But Does Not Affect cAMP Generation. The effect of IL-1 on the cAMP generation by forskolin, a diterpene that directly stimulates adenylate cyclase (19), was studied. IL-1 by itself did not stimulate cAMP nor did it increase accumulation of cAMP induced by forskolin or CRF (Table 1). In contrast, IL-1 pretreatment for 23 hr resulted in an enhanced β-endorphin release by forskolin in a dose-dependent manner (Fig. 4).

IL-1 Potentiates Phorbol Ester (TPA)-Induced β-Endorphin Secretion. The action of IL-1 on the capacity of TPA to induce β-endorphin release was examined. After a 60-min incubation

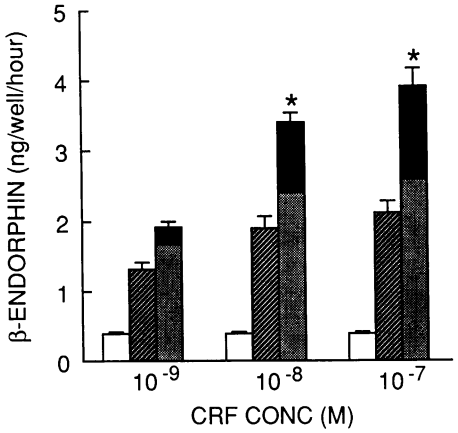

FIG. 3. IL-1 pretreatment potentiates CRF-induced β-endorphin secretion. AtT-20 cells were incubated in presence or absence of 1 nM IL-1α for 23 hr and then washed twice with serum-free medium. The untreated cells were incubated for an additional hour either with vehicle or with various concentrations of CRF (hatched bars). The cells pretreated with IL-1 were also divided into two groups after washing. One group was incubated with IL-1α alone (open bars) and another group with IL-1α plus various concentrations of CRF (stippled bars). After 1 hr, medium was collected from each well and β-endorphin released into the medium was determined. Solid bars represent the enhancement produced by IL-1 on CRF-induced β-endorphin secretion over the additive effect of secretagogues individually. The data (mean ± SEM) are representative of six experiments with similar results. $*P < 0.05$ compared to the additive effects of IL-1α and CRF separately incubated.

Table 1. IL-1 enhances forskolin-induced β-endorphin secretion by a mechanism independent of cAMP

Treatment	cAMP, pmol per well per 30 min
Control	85 ± 4.53
CRF (0.1 μM)	153 ± 9.12*
Forskolin (10 μM)	339 ± 29.1*
IL-1	77 ± 8.25
IL-1 + CRF (0.1 μM)	169 ± 15.3†
IL-1 + forskolin (10 μM)	376 ± 32.2†

Cells were incubated with or without 1 nM IL-1α for 23 hr, then washed, and treated as indicated. Values are mean ± SEM from four wells. Similar results were obtained in two other experiments.
*$P < 0.01$ (vs. control).
†Not significantly different compared to the effects of forskolin and CRF alone.

of AtT-20 cells with TPA, the expected increase in β-endorphin secretion was found. After pretreatment with IL-1 for 23 hr, stimulating the cells with TPA plus IL-1 for 1 hr caused more than 100% enhancement in β-endorphin secretion compared to the individual values when added together. The effect was dose-dependent for TPA (Fig. 5 *Upper*). Phorbol ester exerts its action through protein kinase C and its effect is abolished after prolonged incubation with TPA (15). To test whether the enhancing effect of IL-1 on the ability of TPA to induce β-endorphin secretion is mediated by an increase in protein kinase C activity, some cells were further incubated with TPA alone and TPA plus IL-1 for 9 hr. After prolonged treatment with phorbol ester, the secretion induced by phorbol ester was abolished as well as the enhancement induced by IL-1. However, prolonged treatment with phorbol ester had no effect on IL-1-induced β-endorphin secretion (Fig. 5 *Lower*). Similar results were obtained with IL-1β (data not shown).

DISCUSSION

Our study demonstrates that IL-1 directly stimulates β-endorphin release in AtT-20, a mouse anterior pituitary tumor cell line. This effect was apparent only after incubation for at least 18 hr, IL-1α and IL-1β being equally potent. AtT-20 cells have receptors for IL-1 (20), indicating that the effects of IL-1 are receptor mediated. It has been reported that IL-1 stimulates β-endorphin release in primary culture of normal rat anterior pituitary cells only after prolonged incubations (9). In view of these observations, it appears that the contradictory results found by other investigators were due to the shorter periods of treatment. It is likely that IL-1 increases β-endorphin secretion by transmitting a receptor-mediated message to the proopiomelanocortin gene to induce the synthesis of proopiomelanocortin, the precursor for ACTH and β-endorphin.

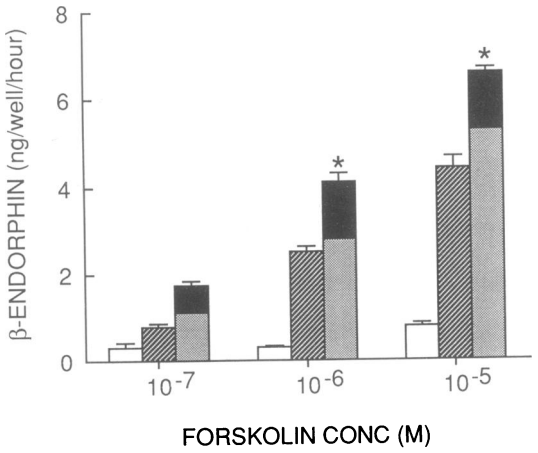

FIG. 4. IL-1 enhances forskolin-induced β-endorphin secretion. AtT-20 cells were pretreated with or without 1 nM IL-1α for 23 hr and then washed and the untreated cells were incubated in serum-free medium for an additional hour either without the test agents or with various concentrations of forskolin (hatched bars). After washing, the IL-1-pretreated cells were treated with serum-free medium either with IL-1 alone (open bars) or IL-1 plus various concentrations of forskolin (stippled bars), and β-endorphin release was measured after 60 min. Solid bars represent the enhancement produced by IL-1 on forskolin-induced β-endorphin release over an additive effect of IL-1 and forskolin separately. The data (mean ± SEM) are representative of three experiments with similar results. *$P < 0.05$; **$P < 0.01$ (vs. the additive effects of IL-1 and forskolin individually).

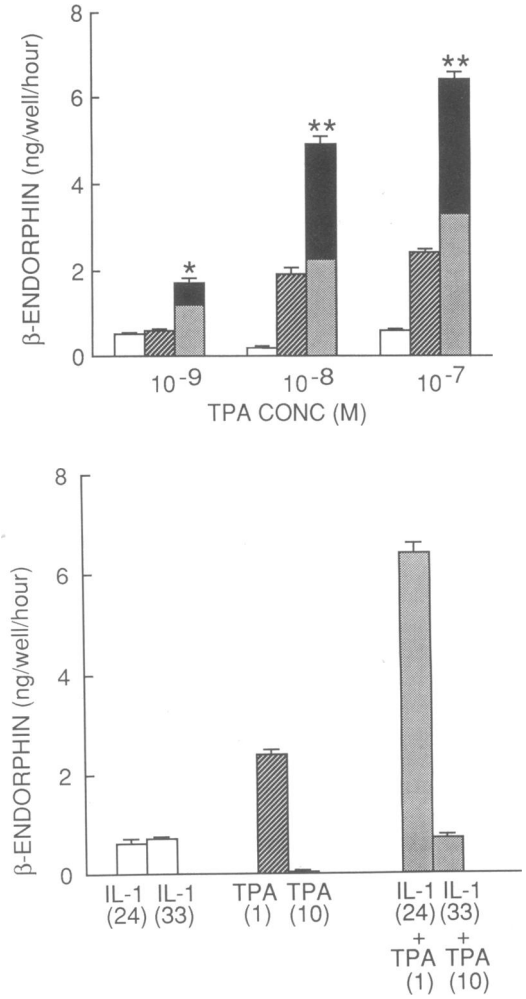

FIG. 5. IL-1 potentiates TPA-induced β-endorphin secretion. (*Upper*) AtT-20 cells were incubated in the absence or presence of 1 nM IL-1α for 23 hr and then washed twice. After washing, the untreated cells were incubated with TPA (hatched bars). IL-1-pretreated cells were treated either with IL-1 alone (open bars) or with IL-1 plus TPA (stippled bars), and the incubation was continued for an additional hour. Aliquots from medium were collected from each well for β-endorphin determination. Solid bars represent the enhancement produced by IL-1 on β-endorphin release induced by TPA. Data are the mean ± SEM of six observations from one of six similar experiments. *$P < 0.05$; **$P < 0.01$ compared to the additive effects of IL-1 and TPA individually. (*Lower*) A portion of the same cells was treated further as follows. Medium was removed by aspiration and fresh medium was added either without the test agent or with TPA alone, IL-1 alone, or IL-1 plus TPA. The incubations continued for 8 hr. After this period of treatment, the cells were washed and treated with TPA alone (hatched bars), IL-1 alone (open bars), or with IL-1 plus TPA (stippled bars), and β-endorphin release was determined after a 60-min incubation. The numbers in parentheses indicate the incubation time in hours. Data are the mean ± SEM of six observations from one of three similar experiments.

IL-1 also increased the effects of other secretagogues, CRF and VIP. After pretreating AtT-20 cells with IL-1 for 23 hr, the secretion induced by CRF and VIP was increased in more than an additive manner. Previous work has shown that IL-1 amplified receptor-mediated formation of prostaglandin E_2 in response to bradykinin, bombesin, and thrombin in 3T3 fibroblasts by increasing the levels of phospholipase A_2 and cyclooxygenase, enzymes involved in prostaglandin E_2 synthesis (21). The treatment with IL-1 was found to cause a 2- to 3-fold increase in the density of β-adrenergic receptors in A549 human lung adenocarcinoma cells (22). The treatment with IL-1 also prevented the loss of corticotrophic response to β-adrenergic stimulation in rat primary pituitary cell culture (23). Our findings, as well as those cited, indicate that IL-1 can amplify a variety of receptor-mediated biological responses.

CRF stimulates ACTH secretion by at least two mechanisms involving cAMP-dependent protein kinase and protein kinase C (24, 25). Our experiments showed that IL-1 had no effect on the generation of cAMP. Previous work has shown that forskolin, a compound known to activate adenylate cyclase, also releases ACTH and β-endorphin in AtT-20 cells (12). Treatment of the cells with IL-1 had no effect on forskolin-stimulated cAMP, but enhanced the action of this diterpene on β-endorphin secretion. This would indicate that forskolin causes the secretion of β-endorphin by a mechanism independent of cAMP. Other investigators had shown that forskolin can exert its effects by direct interaction with K^+ channels (26). Short-time treatment with IL-1 had no effect on adenylate cyclase in AtT-20 cells, suggesting that cAMP is not involved in the mechanism by which IL-1 potentiates the secretion of β-endorphin induced by secretagogues.

Phorbol esters have been found to induce ACTH and β-endorphin release in AtT-20 cells (15). Since phorbol esters act by stimulating protein kinase C, it was suggested that their action on the β-endorphin secretion is mediated by this kinase. Numerous reports had shown that the effects of phorbol esters are desensitized after prolonged incubations (27). Further evidence for such a mechanism was the finding that the stimulatory action of CRF on β-endorphin release was partly abolished by prolonged pretreatment of AtT-20 cells with phorbol ester (28). We found that IL-1 enhances phorbol ester-induced β-endorphin secretion, presumably by acting on protein kinase C. This effect was abolished by prolonged treatment with phorbol ester. Prolonged treatment with phorbol ester had no effect on IL-1-induced β-endorphin secretion. These observations would suggest that the mechanism by which IL-1 enhances the secretory effects of CRF and VIP is mediated by increasing the activity and/or synthesis of protein kinase C.

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