Effect of TNF-α on Prolactin Secretion from Rat Anterior Pituitary and Dopamine Release from the Hypothalamus: Comparison with the Effect of Interleukin-1β

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Abstract. The effects of human recombinant interleukin-1β and -6 and tumor necrosis factor-α (TNF-α) on the releases of PRL and dopamine were examined using monolayer cultures of rat pituitary cells and hypothalamic cells. The release of PRL from rat pituitary cells in 30 min was increased about 2-fold (p<0.05) by 10^5 U/l interleukin-1β, 10^5 U/l interleukin-6 or 100 ng/l TNF-α. TNF-α at 100 ng/l significantly increased PRL release within 5 min incubation and this effect continued throughout the next 30 min of incubation. Incubation for 5 min with TNF-α caused dose-dependent stimulation of PRL release. These cytokines did not modulate [3H]-dopamine release from primary cultures of hypothalamic cells. These results suggest that these cytokines stimulate PRL release directly at the pituitary gland, without modifying the release of dopamine from the hypothalamus.

Key words: Interleukin-1β, Interleukin-6, Tumor necrosis factor-α, Prolactin, Dopamine.

THERE IS accumulating evidence for a close linkage between neuroendocrine and immune functions. Interleukin-1β (IL-1β) and IL-6, which are produced by macrophages or monocytes, directly stimulate the release of multiple hormones from the pituitary gland [1-4]. Recently, tumor necrosis factor-α (TNF-α) was reported to cause direct stimulation of ACTH secretion from the pituitary gland in vitro [5] and in vivo [6]. There are also reports that these cytokines stimulate hormone secretion in a prostaglandin E2- and cAMP-independent manner [3, 7]. Moreover IL-1β has been shown to stimulate secretion of corticotropin releasing factor (CRF) [8, 9], but not of dopamine (DA) [10] from the hypothalamus. Rivier and Vale [11] and others [12] also have reported that human IL-1α and IL-1β act within the brain to inhibit LH secretion. Recently we also found that IL-1β and TNF-α stimulated the release of gonadotropin releasing hormone from dispersed hypothalamic cells [13]. These reports suggest the possibility that these cytokines may act on both the pituitary and hypothalamus.

In this work we examined the effect of IL-1β, IL-6 and TNF-α on the secretion of PRL from monolayer cultures of rat anterior pituitary cells and on dopamine release from dispersed hypothalamic cells.

Materials and Methods

Cultures of pituitary and hypothalamic cells
Female Wistar rats (200–220 g) were decapitated, and the median eminence (containing dopamine neurons) was isolated with fine scissors under a stereomicroscope according to the description of Cuello et al. [14]. Other methods were as described previously [15]. The cells were seeded into Falcon 24-well plates (Falcon, Oxnard, CA) at a density of 1.0×10^5 viable cells/well. Hypothalamic cells were
used after culture for 7 days. Primary monolayer cultures of rat anterior pituitary cells were prepared as described previously [16]. The cells were seeded into Falcon 24-well plates at a density of $0.2 \times 10^6$ viable cells/well allowed to attach for at least 4 days in a humidified 37°C atmosphere of 5% CO$_2$ and 95% air before an experiment was performed.

**Stimulation experiments**

1) PRL release

After culture for 5 days, the anterior pituitary cells were incubated for 3 h in medium RPMI with two changes of the medium. Then they were incubated for 30 min in 1.0 ml of RPMI containing a test material (IL-1β, IL-6, TNF-α or TRH). After incubation, the medium was collected from each well and stored at −80°C until assayed for each pituitary hormone. The viability of the cells after each experimental protocol was consistently greater than 95%, as determined by trypan blue exclusion.

2) Dopamine release

$[^3]$H) Dopamine release from the hypothalamic cells was measured as described previously [15]. Briefly, after culture for 7 days, the cells were preincubated for 120 min in medium TCM-199 containing $[^3]$H) dopamine (3, 4-[7-$^3$H]-dihydroxyphenylethylamine, 37 KBq/ml: New England Nuclear, Boston, MA). They were then washed four times with TCM-199 and incubated in the same medium containing test materials for 30 min. The radioactivity of the incubation medium was then determined in a liquid scintillation counter.

**PRL RIA**

PRL concentrations were determined by the protocol and reagents provided by NIADDK Rat Pituitary Hormone Distribution Program. Results are expressed in terms of NIADDK rat RP-2. The intra- and inter-assay coefficients of variance were less than 6% and 10%, respectively.

**Drugs**

Recombinant human IL-1β, recombinant human TNF-α and TRH were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan, Dainippon Pharmaceutical Co., Tokyo, Japan, and Tanabe Pharmaceutical Co., Osaka, Japan, respectively. Recombinant human IL-6 was prepared by Dr. Toshio Hirano (Osaka, Japan). One unit of IL-6 was defined as the amount required to increase the production of immunoglobulin by the CESS cell line to half the maximal level. One unit of IL-6 is equivalent to 0.2 ng/ml. When the purity of IL-6 is more than 95%, and the preparation has no cytotoxicity, its examination by the neutralization test is unnecessary. The purity of the TNF-α used was more than 99.9%. Calcium ionophore A23187 (Sigma) was dissolved in 100% dimethylsulfoxide (DMSO) and then diluted to the desired concentration with TCM 199 medium. The maximum concentration of DMSO in the culture medium was 0.25%. This concentration did not affect the release of $[^3]$H)dopamine from control cells.

**Statistical analysis**

Data are expressed as nanograms of PRL per well. Each experiment was repeated two or more times to ascertain the reliability of the results. In this study each point is presented as the mean±SEM. All data were subjected to analysis of variance and differences between groups were assessed by the multiple range test of Duncan and a p value of less than 0.05 was considered to represent a significant statistical difference.

**Results**

Effect of TNF-α on PRL release

The dose response of TNF-α-induced release of PRL is illustrated in Fig. 1. Incubation for 5 min

![Fig. 1](image-url)
THE EFFECT OF TNF-α ON PRL AND DA RELEASE

Fig. 2. Time dependent effect of TNF-α (100 μg/l) on release of PRL from rat anterior pituitary cells between 5 and 180 min after its addition. Values arc means ± SEM for concentrations of PRL in the medium. Asterisks indicate significant increase (p<0.05) over the control value.

Fig. 3. Effect of IL-1β (10^5 U/l), IL-6 (10^5 U/l), TNF-α (100 μg/l) and TRH (10^-7 mol/l) on release of PRL from rat anterior pituitary cells in 30 min. Values are means ± SEM for concentrations of PRL in the medium. Asterisks indicate significant increases (p<0.05) over the control value.

with TNF-α caused dose-dependent stimulation of PRL release. The effect of 1000 μg/l TNF-α on PRL release was almost equivalent to that of 10^-7 M TRH. 100 μg/l TNF-α significantly (p<0.05) increased the release of PRL within 5 min of incubation and this effect continued throughout the next 30 min of incubation (Fig. 2).

Effect of IL-1β, IL-6 and TNF-α on PRL release

The effect of IL-1β, IL-6 TNF-α and TRH on PRL release is illustrated in Fig. 3. The effects of 10^-7 M TRH on PRL release was equivalent to that of 10^5 U/l (1 ng/ml) IL-1β, 10^5 U/l (20 ng/ml) IL-6 and 100 μg/l TNF-α.

Effect of IL-1β, IL-6 and TNF-α on release of [³H]dopamine

The effect of IL-1β, IL-6 and TNF-α and calcium ionophore (A23187) on the release of [³H]dopamine from rat hypothalamic cells for 15 min is illustrated in Fig. 4. These three cytokines had no effect on [³H]dopamine release, while calcium ionophore (5×10^-5 mol/l) significantly stimulated [³H]dopamine release.

Discussion

In the present study we clearly showed that TNF-α stimulated the release of PRL from primary cultures of rat anterior pituitary cells in a dose- and time-dependent manner, but that TNF-α did not modulate the release of dopamine from hypothalamic cells. The stimulatory effect of TNF-α on the release of PRL was rapid, being observed within 5 min, but no longer evident after 60 min. IL-1β and IL-6 also stimulated PRL release, but these three cytokines did not modify dopamine release.

IL-1β and IL-6 are known to stimulate PRL release [1–4] but there is no previous report of the stimulation of PRL release by TNF-α. The effect of TNF-α on pituitary hormone release is con-
We [17] and others [5, 6] have reported the stimulatory effects of TNF-α on rat anterior pituitary hormones, while other [18, 19] reported inhibitory effects. Moreover, Milenkovic et al. [5] reported that TNF-α stimulates the release of ACTH, GH and TSH but not of PRL release in vitro. They examined the effect of TNF-α after its incubation with pituitary cells for 2 h, but not after a shorter culture period, such as that used in our study. Sharp et al. [6] also reported that TNF-α stimulated ACTH release in vivo but not in vitro, but they only examined the effect of TNF-α on pituitary cells in a 4 h culture period. In addition, inhibitory effects of TNF-α were demonstrated only after 24 h of incubation [18, 19]. On the other hand, stimulatory effects of TNF-α were observed in PRL release within 5 min in our study. Therefore the discrepancy in the reports to date may be at least in part due to differences in the experimental design especially with regard to the incubation time.

It is now well documented that a paracrine control mechanism exists in the anterior pituitary. Recently we have observed that TNF-α stimulated the production of IL-6 [17] and release of arachidionate from dispersed anterior pituitary cells (submitted for publication), indicating that these products might have a local modulatory action on TNF-α-induced PRL release. Taken together, our recent findings lead to the speculation that the TNF-α-activated cells may release both acute stimulatory and latent inhibitory signals, although the mechanism by which TNF-α acts in both acute and delayed situations remains unclear.

TNF-α damages a number of tumor cells in vivo and in vitro, but it has little or no toxic effect on normal cells [20, 21]. We found by the dye exclusion test that in our conditions TNF-α did not affect the viability of the cells. Toxicity assessment of TNF-α on pituitary cells has been also performed by Walton et al. [18] and others [5, 6]. With a calcium-sensitive fluorescent dye and a digital imaging fluorescence microscopic system, we also observed that after the TNF-α-induced increase, \([Ca^{2+}]_i\) returned to nearly the basal level after 120 sec, indicating that this cytokine does not have a toxic effect on normal pituitary cells (submitted for publication).

It has been reported that IL-1β and IL-6 stimulate ACTH secretion through a stimulatory effect of CRF [8, 9], but IL-1β did not modify dopamine release [10] from the hypothalamus. Other investigators also have shown that IL-1β, IL-6 and tumor necrosis factor all lowered plasma LH levels in castrated rats after intracerebroventricular injection, suggesting that these cytokines may modulate LH secretion through the inhibition of GnRH secretion, either directly at the hypothalamic level [12] or through the release of endogenous CRF and/or opiates [22]. In contrast to this, we have found that IL-1β and TNF-α stimulated the release of GnRH [13]. Taken together, these reports suggest the possibility that these cytokines may also act directly at the hypothalamic level. Further studies on the effect of cytokines on other PRL modulating compounds in the hypothalamus are necessary.

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References


