The Effect of Interleukin-2 on the Release of Gonadotropin and Prolactin *in Vivo* and *in Vitro*

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Abstract. To evaluate a possible physiological role of Interleukin-2 (IL-2) in the control of Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and Prolactin (PRL) release, conscious, ovariectomized (OVX) rats were given injections of IL-2 into the third ventricle. The third ventricular injection of IL-2 induced a significant decrease in plasma LH levels when compared to values in control animals ($P<0.05$). Plasma LH concentrations were significantly decreased within 5 min after the injection of IL-2 and remained decreased for 1 h. In contrast, injections of IL-2 had no effect on plasma FSH or PRL levels. To evaluate a possible direct action of IL-2 on LH, FSH and PRL release from the anterior pituitary gland, the cytokine was incubated with dispersed anterior pituitary cells for 4 h. IL-2 in the dose range between 10^{-1} and 10^{-3} unit stimulated the release of LH and FSH into the culture medium ($P<0.05$, $P<0.025$ vs. control, respectively). The release of PRL from incubated anterior pituitary cells was not affected at any dose of IL-2 tested. These results indicate that IL-2 possibly plays an inhibitory role in the control of gonadotropin secretion, via hypothalamic action, although it acts directly to stimulate the release of the gonadotropins at the level of the anterior pituitary gland.

Key words: Interleukin-2 (IL-2), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Prolactin (PRL), Intraventricular (third ventricle) injection, Dispersed anterior pituitary cells

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RECENT experimental evidence has established the fact that the neuroendocrine and immune systems are intimately linked and involved in bidirectional communication [1]. The immune system and its products can modulate neuroendocrine functions and neuroendocrine hormones have been seen to affect the immune response. The interplay between these systems is believed to be mediated by cytokines. Interleukin-1 (IL-1), a cytokine secreted by stimulated macrophages and monocytes, is able to control neuroendocrine functions and has been localized within the central nervous system [2–8]. Interleukin-6 (IL-6) has been

also reported to regulate anterior pituitary hormone secretion [9, 10] and high affinity IL-6 binding sites were found in the bovine hypothalamus [11]. The anterior pituitary gland was reported to produce IL-6, which may act as an intrapituitary releasing factor [11, 12, 14]. These findings indicate that interleukins are involved in the control of anterior pituitary hormones at both the hypothalamic and pituitary levels.

IL-2 is a small (15.5 kDa), single-chain protein that enhances T-lymphocyte proliferation after antigenic stimulation. Recent evidence has confirmed the existence of IL-2 immunoreactivity and IL-2-binding sites in the rat central nervous system [15, 16]. IL-2 was also reported to affect the release of anterior pituitary hormones at the level of the pituitary gland [17]. It is therefore not surprising that IL-2 may play a physiological role in the control of anterior pituitary hormone secretions at the

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level of the hypothalamus and/or pituitary gland, but the physiological actions and its biological activities in the brain have not been clearly understood. The present studies were designed to evaluate the role of IL-2 in the control of LH, FSH and PRL secretion by injecting it into the third ventricle and by also incubating it with dispersed anterior pituitary cells *in vitro*.

Materials and Methods

Materials

Adult female Sprague-Dawley rats weighing 250–300 g were used. They were housed in group cages under conditions of controlled temperature (22–25°C) and lighting (on from 0500 h to 1900 h) and provided with rat chow and water *ad libitum*. All animals were used 3–5 weeks after ovariectomization (OVX). OVX was performed under ether anesthesia. Antibiotics were injected immediately after OVX. Rat IL-2 (recombinant, 1 BRMP unit=0.058 ng, Becton Dickinson Labware) was used.

In vivo experiments

Seven days before the experiment, a 23-gauge stainless steel cannula (17 mm in length) was implanted in the third ventricle of OVX rats as described earlier [18]. A mandril was inserted into each cannula to prevent its obstruction. The location of the cannula in the third ventricle was confirmed by the continuous flow of cerebrospinal fluid from the implanted cannula. After implantation, the animals were housed in individual cages until the day of the experiment. Twenty-four hours prior to the experiment, an indwelling catheter was inserted into the right jugular vein while the animal was anesthetized with ether as described earlier [19]. On the morning of the experiment, various doses of IL-2 dissolved in a volume of saline or an equal volume of physiological saline were injected into the third ventricle after the animal was left undisturbed for more than 60 min. Blood samples were withdrawn at various time intervals thereafter. Each blood sample was immediately replaced by an equal volume of heparinized physiological saline. Plasma was separated by centrifugation at 4°C and stored frozen until the

day of the assay.

In vitro experiments

Pituitary glands were collected after decapitation. The posterior lobes of pituitary glands were removed and the anterior lobes were dispersed by a modified method of Vale *et al.* [20]. The dispersed cells were aliquoted (2.5×10^5 cells/ml) into 24-well tissue culture plates and preincubated at 37°C under 5% CO₂-95% air in Earle's medium 199 (M 199, Gibco) containing 25 mM Hepes, 10% horse serum, penicillin and streptomycin. After 3 days culture the cells were washed twice with 1 ml of Earle's medium 199 containing 25 mM Hepes and 0.3% bovine serum albumin (BSA fraction V, Sigma Chemical Co.). IL-2 were diluted to various concentrations and cells were incubated in 1 ml of the medium containing IL-2. The incubation was continued for 4 h at 37°C under 5% CO₂-95% air. At the end of the incubation, the medium was stored at –80°C until the day of the assay. To exclude the possibility of a toxic effect of the applied substance, the viability of the cultured cells was assayed by the dye-exclusion method. Viability was always over 90% both before and after the incubation.

Hormone assays

Plasma LH, FSH and PRL were measured by specific radioimmunoassay with the kits supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Results were expressed in terms of NIDDK-rat-LH-RP-2, FSH-RP-2 and PRL-RP-1 reference preparations.

Differences from the starting values in each group studied were analyzed by a one-way analysis of variance followed by the Student-Newman-Keuls multiple comparison test.

Results

Effect of intraventricular administration of IL-2

Figure 1 illustrates the effect of intraventricular administration of IL-2 on LH release. The higher dose of IL-2 (40 unit) significantly suppressed LH release compared to those in the saline-injected control group. The effect was observed within 5

min after injection and levels remained suppressed for 60 min. Little change in plasma LH was observed after intraventricular injection of lower dose of IL-2 (4 unit).

None of the IL-2 doses injected into the third ventricle induced significant changes in plasma FSH and PRL levels in OVX rats (Figs. 2, 3).

In vitro studies

IL-2 produced a dose-dependent increase in LH

Fig. 1. Effect of intraventricular injection of IL-2 on LH release in OVX rats. Because of a variation in initial values between groups, results are presented as the change in plasma LH at various times after the injection (Δ LH) for greater clarity. In this and subsequent figures, the values obtained are given as the mean $\pm$ SEM. n=Number of samples. The initial values prior to the injections were 7.49 ± 0.41 ng/ml for the control, 10.24 ± 1.11 ng/ml for the IL-2 (40 unit)-injected rats and 6.31 ± 0.72 ng/ml for the IL-2 (4 unit)-injected rats.

Fig. 3. Effect of intraventricular injection of IL-2 on PRL release in OVX rats.

release in the dose-range between 10^{-1} to 10^{-3} unit, with significant differences from control incubations for all doses tested ($P < 0.005$; Table 1). IL-2, in the dose range between 10^{-1} – 10^{-3} unit, also stimulated the release of FSH into the culture medium from dispersed anterior pituitary cells ($P < 0.005$; Table 2). In contrast, IL-2 failed to cause any changes in PRL secretion (Table 3).

Fig. 2. Effect of intraventricular injection of IL-2 on FSH release in OVX rats.

Table 1. Effect of IL-2 on LH release from dispersed anterior pituitary cells (n=4)

Treatment	LH release (ng/ml $\cdot$ 10^6 cells $\cdot$ 4 h)
Control	1.497 ± 0.103
10^{-1} u/ml IL-2	4.575 ± 0.868^1
10^{-2} u/ml IL-2	3.587 ± 0.246^2
10^{-3} u/ml IL-2	2.698 ± 0.171^2

Values are mean $\pm$ SEM. ¹, $P < 0.05$; ², $P < 0.005$.

Table 2. Effect of IL-2 on FSH release from dispersed anterior pituitary cells (n=4)

Treatment	FSH release (ng/ml $\cdot$ 10^6 cells $\cdot$ 4 h)
Control	0.359 ± 0.013
10^{-1} u/ml IL-2	0.453 ± 0.015^1
10^{-2} u/ml IL-2	0.410 ± 0.006^2
10^{-3} u/ml IL-2	0.502 ± 0.023^1

Values are mean $\pm$ SEM. ¹, $P < 0.005$; ², $P < 0.025$.

Table 3. Effect of IL-2 on PRL release from dispersed anterior pituitary cells (n=4)

Treatment	PRL release (ng/ml•10 ⁶ cells•4 h)
Control	92.3 ± 1.5
10 ⁻¹ u/ml IL-2	91.8 ± 2.8
10 ⁻² u/ml IL-2	88.5 ± 2.5
10 ⁻³ u/ml IL-2	97.3 ± 3.8

Values are mean ± SEM.

Discussion

The results of the present study demonstrate that IL-2 injected into the third ventricle suppresses the release of LH in unanesthetized, freely moving OVX rats, suggesting its hypothalamic inhibitory action on LH release. In contrast, IL-2 directly stimulates LH and FSH release from the dispersed anterior pituitary cells into the incubation medium *in vitro*. IL-2 had no effect on PRL release *in vitro* or *in vivo*.

Recent studies have identified the existence of IL-2 immunoreactivity and IL-2 receptors in the rat brain and human brain as well [15, 16, 21]. In a series of *in vitro* experiments on Wistar rats, it was reported that IL-2 injected into the third ventricle induced a significant change in neuronal firing [22]. There was a decrease in the neuron discharge frequency in the ventromedial nucleus of the hypothalamus and an increase in the supraoptic and paraventricular nuclei. Furthermore, Nistico and Sarro reported that IL-2 produced dose-dependent behavioural soporific effects and increases in EEG spectrum power when injected into the third ventricle in rats [15]. They suggested that opiate and IL-2 receptors might be functionally coupled because naloxone, an antagonist at opioid receptors, was able to antagonize the central effects of IL-2. These findings suggest that the endogenous opioid peptides (EOP) may be involved in the modulation of IL-2 effects in the central nervous system. In recent years it has been made increasingly evident that interleukins interfere with reproductive function. Rivier and Vale reported that central administration of IL-1 into the lateral ventricle of castrated rats inhibited LH release [4]. Kalra *et al.* also reported that IL-1 administered intracerebroventricularly blocked the progesterone-induced LH surge and IL-1 suppressed LHRH re-

lease *in vitro*, suggesting that suppression of the steroid-induced LH surge by IL-1 may be due to the inhibition of LHRH at hypothalamic sites. They suggested that the inhibitory effect of IL-1 on LHRH-LH release was mediated by EOP systems [23]. It is not surprising that the inhibitory effect of IL-2 on LH release may also be mediated by EOP systems because IL-2 exerts its central effects via EOP systems as described earlier. The failure of FSH release to decrease following the intraventricular injection of IL-2 in our study is probably accounted for by the relative insensitivity of FSH release as compared to that of LH [18].

There is increasing evidence that interleukins directly act at the pituitary level to modulate the release of anterior pituitary hormones [1, 7, 8, 12, 17]. Numerous *in vitro* studies have addressed the direct effects—with confusing results [1]. IL-1 has been shown to stimulate the secretion of LH, ACTH, GH and TSH [7, 8]. IL-1 was reported to stimulate PRL release [8] and inhibit the hormone [7]. The release of LH, GH and PRL from dispersed anterior pituitary cells was stimulated by IL-6 [24]. In contrast, IL-6 failed to alter LH, FSH and PRL release from incubated hemipituitaries [10]. Karanth and McCann reported the ability of IL-2 to modulate the release of anterior pituitary hormones by a direct action on hemipituitaries incubated *in vitro*. They indicated that IL-2 augmented the basal release of PRL, TSH and ACTH and attenuated the basal release of LH, FSH and GH [17]. In the present study, we demonstrate the opposite effect of IL-2. The reason for the discrepancies between their results and ours is still unclear; however, we used rat IL-2 instead of human IL-2 and dispersed anterior pituitary cells of OVX rats instead of male rat hemipituitaries as an *in vitro* model. These differences could explain the discrepancy between the two reports.

Our present results indicate the hypothalamic inhibitory action of IL-2 and its direct stimulatory action at the pituitary level on gonadotropin release, which is similar to IL-1 action. Our results on the direct IL-2 effect on FSH release (Table 2) did not show dose-dependency. Further studies are therefore necessary to determine the minimum effective dose of IL-2 on gonadotropin release in order to clarify its physiological role on the hypothalamic-pituitary axis. However, the results provide further evidence of a putative functional link between the immune and neuroendocrine systems.

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