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Anterior pituitary hormone control by interleukin 2

(hemipituitary incubation/corticotropin/prolactin/growth hormone/potassium-induced release)

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ABSTRACT **Several monokines, proteins secreted by monocytes and macrophages, alter release of hormones from the anterior pituitary. We report here the ability of femtomolar concentrations of interleukin 2 (IL-2), a lymphokine released from T lymphocytes, to alter directly pituitary hormone release. The effects of concentrations of IL-2 ranging from 10^{-17} to 10^{-9} M on anterior pituitary hormone release were evaluated *in vitro*. Hemipituitaries were preincubated in 1 ml of Krebs–Ringer bicarbonate buffer (KRB) followed by incubation for 1 or 2 hr with KRB or KRB containing different concentrations of IL-2. This was followed by incubation for 30 min in 56 mM potassium medium to study the effect of pretreatment with IL-2 on subsequent depolarization-induced hormone release. Prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), corticotropin (ACTH), growth hormone (GH), and thyrotropic hormone (TSH) released into the incubation medium were measured by radioimmunoassay. IL-2 stimulated the basal release of PRL at 1 or 2 hr but suppressed the subsequent depolarization-induced PRL release, perhaps because the readily releasable pool of PRL was exhausted. The minimal effective dose (MED) was 10^{-15} M. Conversely, IL-2 significantly suppressed the basal release of LH and FSH at 1 or 2 hr, with a MED of 10^{-16} M, thus demonstrating a reciprocal action of the cytokine on lactotrophs and gonadotrophs. The subsequent depolarization-induced release of LH and FSH was suppressed, indicative of a persistent inhibitory action of IL-2. IL-2 stimulated ACTH and TSH release at 1 hr and the MEDs were 10^{-12} and 10^{-15} M, respectively. Conversely, IL-2 significantly lowered the basal release of GH at 1 hr, with a MED of 10^{-15} M. The release of GH was not altered at 2 hr. The high potassium-induced release of ACTH, TSH, and GH was not affected. The results demonstrate that IL-2 at picomolar concentrations affects the release of anterior pituitary hormones. This cytokine may serve as an important messenger from lymphocytes exerting a direct paracrine action on the pituitary by its release from lymphocytes in the gland or concentrations in the blood that reach the gland may be sufficient to activate it.**

Bidirectional intercellular communication between the neuroendocrine and immune systems is firmly established (1, 2). The interplay between these systems is believed to be mediated by proteins termed cytokines (3). Interleukin 2 (IL-2) is a lymphokine, synthesized and secreted by T lymphocytes activated by antigen or mitogen (4, 5). It is a glycoprotein (15.5 kDa) that stimulates the proliferation of T cells, B cells, natural killer cells, and lymphocyte-activated killer cells (3). IL-2 has been reported to enhance the expression of the proopiomelanocortin (POMC) gene by cultured normal and pituitary tumor (AT-20) cells (6). A similar activation of POMC mRNA has already been demonstrated for IL-1 (7).

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IL-2 increased plasma corticotropin (ACTH) and glucocorticoid levels when injected into patients with cancer or acquired immunodeficiency syndrome (8). This suggests that IL-2 may increase ACTH release either by increasing corticotropin-releasing factor release from the hypothalamus or by a direct stimulation of the release of ACTH from the anterior pituitary (AP) gland. The objective of this study was to evaluate the possible direct actions of IL-2 on AP hormone release *in vitro* and its effect on subsequent depolarization-induced AP hormone release.

MATERIALS AND METHODS

Adult male rats of the Sprague–Dawley strain (Holtzmann, Madison, WI) weighing 200–250 g were housed under controlled conditions of temperature (24 ± 1°C) and lighting (on 5:00–17:00 hr). The animals were fed a pellet diet with water freely available. After acclimatization for 5 or more days in the vivarium, the rats were killed by decapitation.

IL-2. Human IL-2 (recombinant, catalog no. 40043, *Escherichia coli*, Collaborative Research) was used.

***In Vitro* Incubation.** After removal of the posterior lobe, APs were bisected longitudinally. Two hemipituitaries per tube were preincubated in 1 ml of Krebs–Ringer bicarbonate (KRB) buffer (pH 7.4) in an atmosphere of 95% O_2/5% CO_2 in a Dubnoff shaker (50 cycles per min) for a period of 45 min. The medium was then decanted and the tissue was incubated with 1 ml of fresh KRB (control) or with KRB containing different concentrations of IL-2 (10^{-17} to 10^{-9} M) for 1 or 2 hr. After 2 hr, the medium was aspirated and stored, and the tissue was incubated with 1 ml of fresh KRB containing high potassium (K^+ = 56 mM) for 30 min. At the end of this final incubation, medium was collected and stored. The AP hormones prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), and thyrotropic hormone (TSH) released into the incubation medium were assayed by RIA with kits provided by the National Institute of Diabetes and Digestive and Kidney Diseases. In the case of ACTH, antibodies against human ACTH were purchased from EGG Group (Nashville, TN). Human ACTH (Peninsula Laboratories) was iodinated with ^{125}I and purified on columns of Sephadex G-50, fine grade. Values were expressed in terms of the reference preparations provided with the kits.

Statistics. Since the results from consecutive experiments did not differ significantly from each other, the pooled results from two or three experiments are presented. Statistical probabilities were calculated by using one-way analysis of variance and the Student–Newman–Keuls multiple comparison test for unequal replications. Comparisons resulting in $P < 0.05$ were considered significant.

Abbreviations: IL, interleukin; GH, growth hormone; PRL, prolactin; LH, luteinizing hormone; ACTH, corticotropin; FSH, follicle-stimulating hormone; TSH, thyrotropic hormone; AP, anterior pituitary; MED, minimal effective dose.

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RESULTS

PRL Release. The minimal effective dose (MED) of IL-2 required to activate PRL release after 1 hr of incubation was 10^{-15} M (Fig. 1). Further increases in IL-2 concentration caused no additional increase in PRL release and, when the concentration was increased to 10^{-9} M, release was no longer significantly elevated. After 2 hr of incubation the pattern of release was similar except that the MED was 10^{-14} M.

A marked suppression of depolarization-induced PRL release was observed with all groups pretreated with IL-2 except for the highest concentration (10^{-9} M), which did not stimulate basal release (Fig. 1 *Top*). In most of the treated groups the suppression was ≈40%. The suppression was apparent even at 10^{-17} and 10^{-16} M IL-2, concentrations that did not alter the basal release.

LH Release. In contrast to the results with PRL, IL-2 inhibited LH release with a MED of 10^{-16} M. There was no further increase in the inhibition as the concentrations were increased to 10^{-10} M and inhibition was no longer significant at 10^{-9} M IL-2. The pattern at 2 hr was exactly the same as that at 1 hr except that the inhibition was slightly greater.

Pretreatment with 10^{-15} to 10^{-10} M IL-2 elicited a pronounced decrease in the amount of LH released into the incubation medium by the high [K^+] depolarizing stimulus (Fig. 2 *Top*).

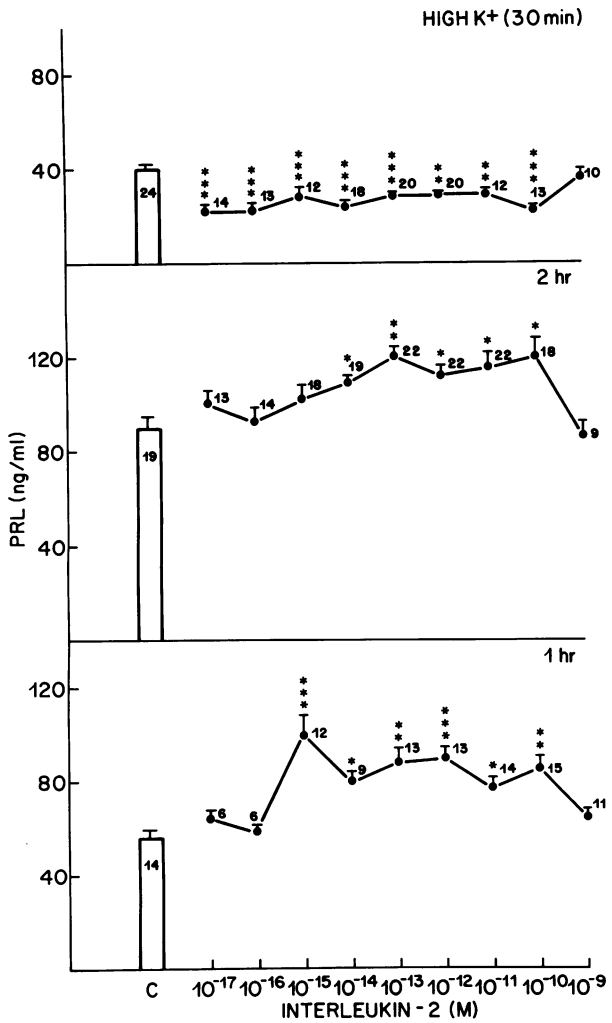

FIG. 1. Effect of IL-2 on PRL release by APs incubated *in vitro* for 1 or 2 hr. In this and subsequent figures, results are expressed as mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; P values versus control, respectively, in this and subsequent figures. The number in each bar represents the number of tubes per group. (*Top*) Release of PRL after incubation with medium containing high [K^+] for 30 min 2 hr after addition of IL-2 or diluent.

FSH Release. The pattern of response of FSH was exactly the same as that for LH with a significant reduction in the amount of FSH released at 1 and 2 hr, with a MED of 10^{-16} M IL-2 (Fig. 3). Again, suppression was no longer significant at the highest dose of 10^{-9} M. In this case the maximal suppression was observed with 10^{-10} M at 1 hr and 10^{-12} M at 2 hr.

In the groups treated with 10^{-14}, 10^{-13}, and 10^{-12} M IL-2, there was a significant decrease in depolarization-induced FSH release (Fig. 3 *Top*).

ACTH Release. Doses of 10^{-12} and 10^{-11} M IL-2 induced a profound increase in ACTH release at 1 hr (Fig. 4). The other doses (10^{-17} to 10^{-13}, 10^{-10}, and 10^{-9} M) exhibited a tendency to increase release, which was not statistically significant. IL-2 failed to alter the release at 2 hr and also failed to alter depolarization-induced ACTH release (Fig. 4 *Top*).

GH Release. The release of GH was suppressed by IL-2, with a MED of 10^{-15} M. The inhibition had disappeared at the higher concentrations tested, 10^{-10} and 10^{-9} M, after 1 hr of incubation (Fig. 5). IL-2 did not alter the release at 2 hr or GH release induced by incubation with medium containing high [K^+] (Fig. 5 *Top*).

TSH Release. IL-2 increased TSH release, with a MED of 10^{-15} M and a maximal effect at 10^{-10} M, which disappeared at the highest concentration tested, 10^{-9} M, following 1 hr of

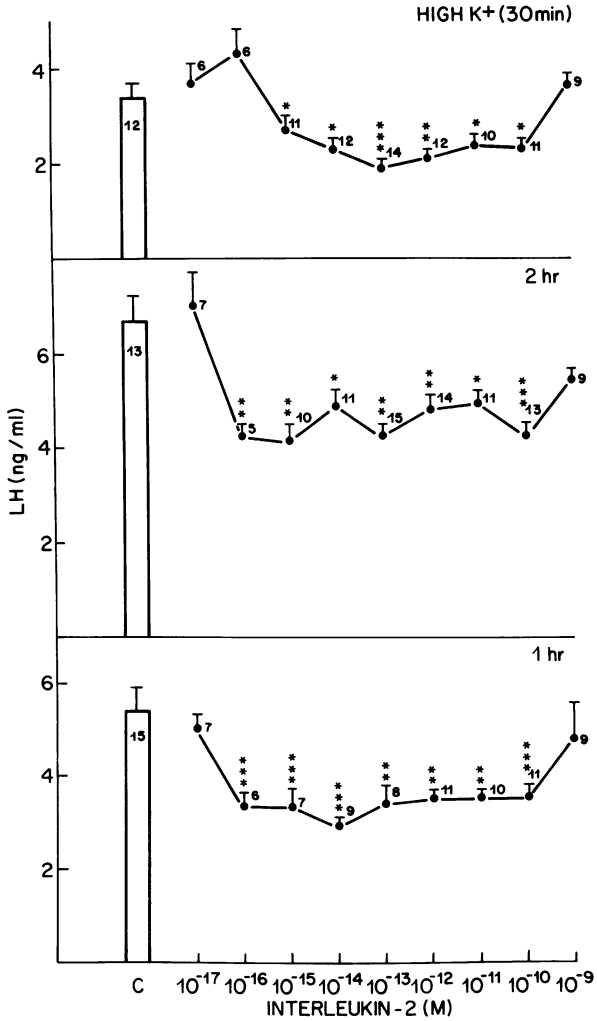

FIG. 2. Effect of IL-2 on LH release by APs incubated *in vitro* for 1 or 2 hr and subsequent release induced by high [K^+].

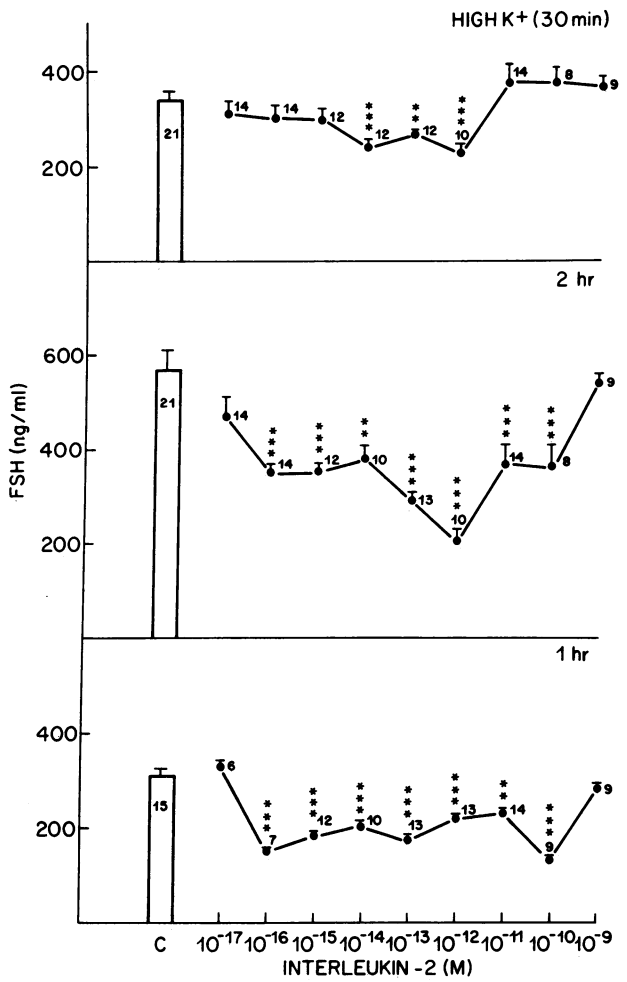

FIG. 3. Effect of IL-2 on FSH release by APs incubated *in vitro* for 1 or 2 hr and subsequent release induced by high [K^+].

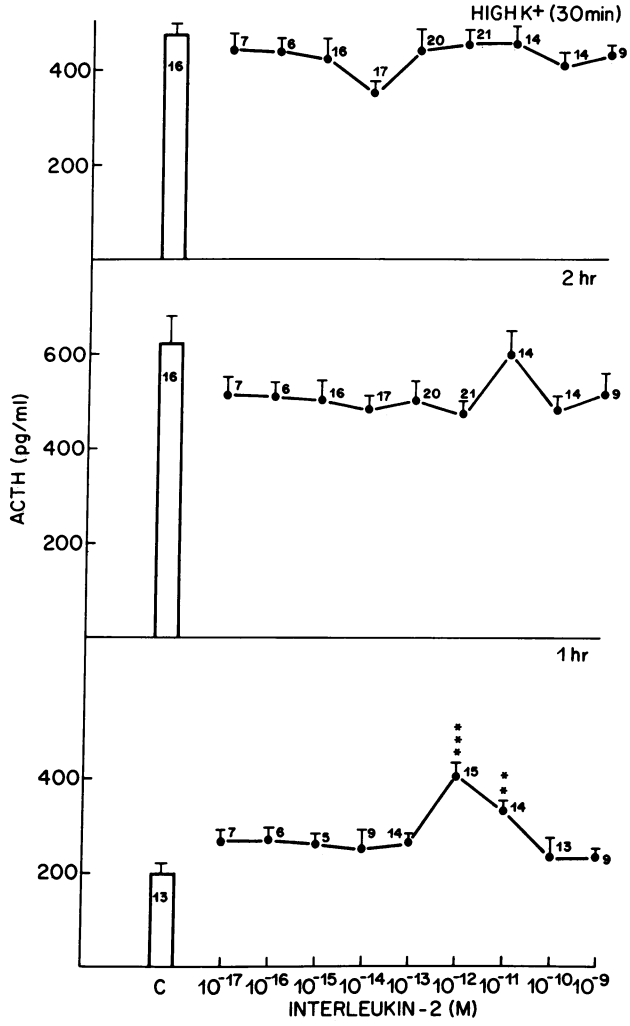

FIG. 4. Effect of IL-2 on ACTH release by APs incubated *in vitro* for 1 or 2 hr and subsequent release induced by high [K^+].

incubation (Fig. 6). However, at 2 hr, there was an opposite inhibitory effect, which was significant only for the 10^{-13} M concentration. High [K^+]-induced TSH release was not altered by any of the concentrations of IL-2 (Fig. 6 *Top*).

DISCUSSION

The results demonstrate the ability of picomolar concentrations of IL-2 to modulate the release of all AP hormones by a direct action on hemipituitaries incubated *in vitro*. IL-2 consistently augmented the basal release of PRL, TSH, and ACTH and consistently attenuated the basal release of LH, FSH, and GH. The concentrations that stimulated (10^{-15} to 10^{-10} M) the release of PRL also markedly suppressed the release of gonadotrophins, thus illustrating a reciprocal relationship between the induction of secretion by lactotrophs and gonadotrophs. The close association of gonadotrophs and lactotrophs to form cup-shaped clusters of cells suggests a functional relationship between these cell types (9, 10). Denef and Andries (11) reported the paracrine interaction between lactotrophs and gonadotrophs in pituitary cell aggregates. They suggested that in the presence of LHRH, gonadotrophs stimulate the secretory activity of lactotrophs through the release of a paracrine humoral factor; however, IL-2 stimulated the lactotrophs and inhibited the gonadotrophs, which raises the possibility that IL-2 stimulated the release of PRL, which had a paracrine action to suppress release of FSH and LH from the gonadotrophs. Alternatively, there may be IL-2 receptors on these two cell types so that IL-2 directly alters hormone release from each cell type. The presence of IL-2 receptors on the various cell types is the most likely explanation for the effects of the lymphokine on the secretion of the other pituitary hormones.

Membrane depolarization by high [K^+] causes hormonal release from endocrine tissues. Elevated [K^+] stimulates the release of PRL from the AP (12, 13). In our study also, a 3-fold increase in PRL release occurred in glands incubated with high [K^+] medium, as compared to those glands incubated with normal [K^+] medium alone. A striking feature was the suppression of PRL release by pretreatment with IL-2. This may be related to depletion of a releasable pool of PRL by the prior stimulation by IL-2.

The stimulation of PRL release by IL-2 is consistent with the postulated role of PRL in the regulation of immune cell function (14). Although PRL is important to reproductive functions, many investigators have reported its involvement in immune regulation (14, 15). PRL receptors are present on normal T and B lymphocytes and monocytes (16, 17) and it regulates γ-interferon production by T lymphocytes (18). A recent report (19) suggests that PRL induces IL-2 cell surface receptors but not IL-2 secretion from rat splenic lymphocytes. IL-6, another product of T lymphocytes, has also been shown to enhance PRL release by pituitary cells (20).

IL-2 suppressed LH and FSH release at extremely low concentrations at 1 and 2 hr. This suppression was observed even after incubation with medium containing high [K^+]. This may be the result of residual suppression of release by the lymphokine.

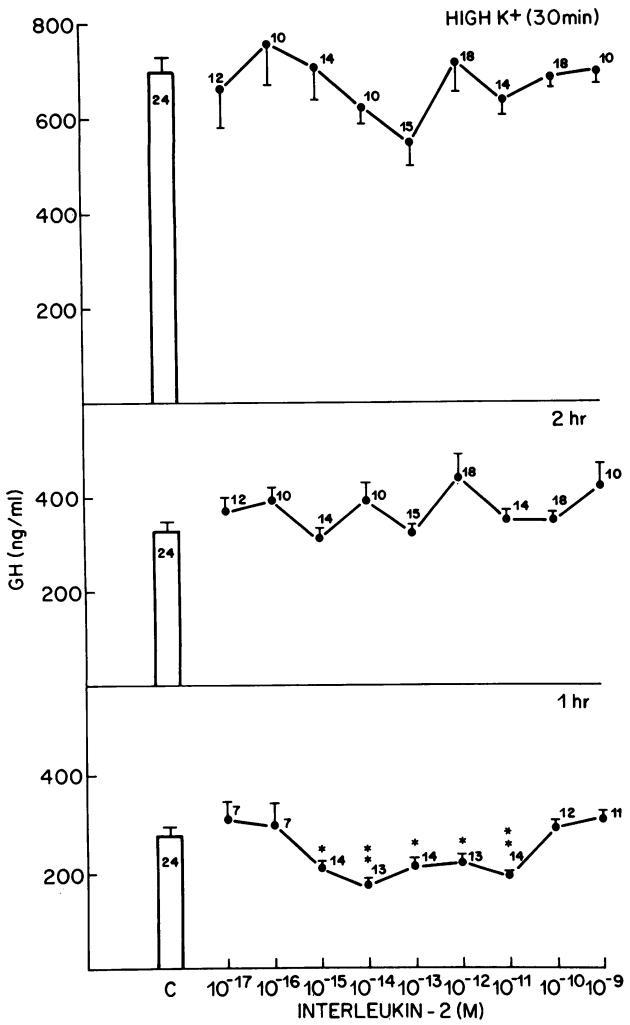

FIG. 5. Effect of IL-2 on GH release by APs incubated *in vitro* for 1 or 2 hr and subsequent release induced by high [K^+].

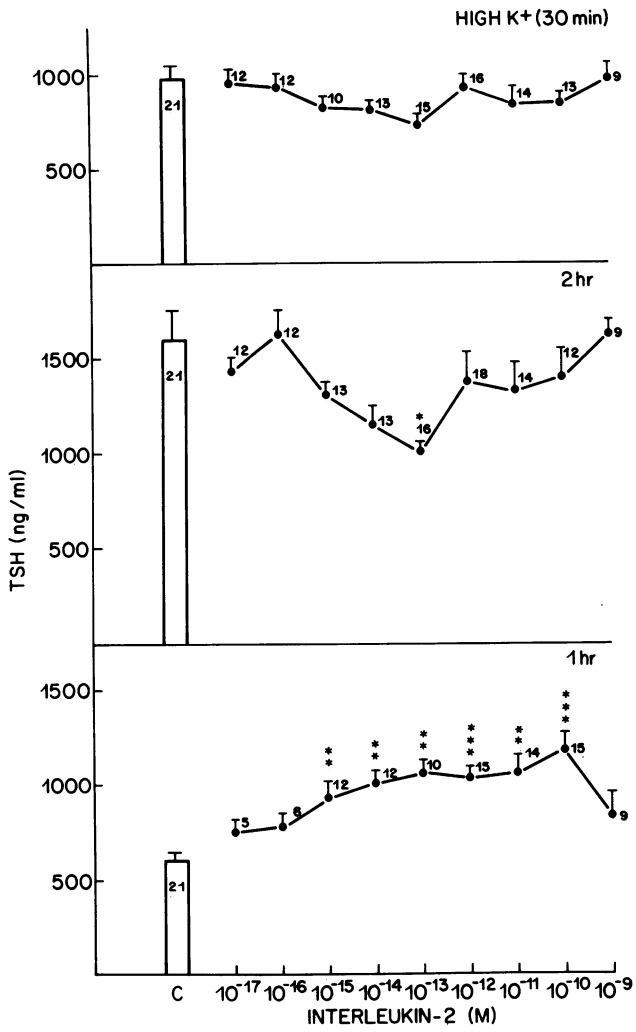

FIG. 6. Effect of IL-2 on TSH release by APs incubated *in vitro* for 1 or 2 hr and subsequent release induced by high [K^+].

Another important observation was the suppression of GH release by IL-2. GH has also been shown to stimulate immune function *in vivo* and *in vitro* (21, 22). The deficient immune response of the hypophysectomized animal can be corrected by the administration of GH (21). In our study an enhanced PRL release and a suppressed GH release were observed. Whether there is any link between these effects is not clear. Investigators have reported the colocalization of PRL and GH in pituitary cells known as mammosomatotrophs, which have been shown to secrete PRL and GH (23). The physiological relevance of these cells and the existence of a possible paracrine influence between lactotrophs and somatotrophs have not been demonstrated.

IL-2 served as a potent stimulus for the release of ACTH; however, the concentration required (10^{-13} M) was greater than for the other pituitary hormones. IL-2 administered to patients with cancer or acquired immunodeficiency syndrome increased plasma ACTH and cortisol concentrations (7), possibly by means of such a direct stimulation of the corticotropes.

Pituitary hormones, including ACTH, endorphin, TSH, and PRL, bind to lymphocytes and modulate their function (24–27). In turn, upon stimulation, lymphocytes produce molecules very similar to the peptide hormones ACTH, endorphin, TSH (28, 29), and LH-releasing hormone (30), thus establishing an important interplay between the neuroendocrine and immune systems.

Since IL-2 is able to modulate the release of all AP hormones at picomolar concentrations, it may be an important immunologic messenger exerting direct action at the pituitary level. In fact, the potency of IL-2 to alter the release of pituitary hormones (MED, 10^{-16} to 10^{-13} M) is in most instances greater than that of the hypothalamic releasing and inhibiting hormones themselves (31–35). It appears probable that the concentrations of the lymphokine that reach the gland by way of the circulation, or possibly by local production within the pituitary itself, play an important role in determining the response of the pituitary to infection. Although concentrations of IL-2 in the circulation during infection are lower than the normal values (36), even these low levels may be effective directly on the AP.

The pattern of AP response to IL-2 is unique among the cytokines since it mimicks almost completely the pattern of AP hormone response to stress, which in the rat consists of increased ACTH and PRL and decreased FSH, LH, TSH, and GH release (37). The only exception is TSH, the release of which is increased by IL-2 instead of being decreased as in stress.

IL-2 may also exert hypothalamic actions to alter release of pituitary hormones. If so, the final pattern of release *in vivo* will depend on interactions between the hypothalamic and direct pituitary influences. The final pattern of pituitary hormone release in infections will also be determined by the actions and interactions with other monokines released dur-

ing infection, which also act at hypothalamic and pituitary levels to alter the release of AP hormones (38–40).

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