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Effects of Interleukin-1β on Thyrotropin Secretion and Thyroid Hormone Uptake in Cultured Rat Anterior Pituitary Cells*

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ABSTRACT

The effects of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) on basal and TRH-induced TSH release, and the effects of IL-1β on the uptake of $[^{125}I]T_3$ and $[^{125}I]T_4$ and on nuclear binding of $[^{125}I]T_3$ were examined. Furthermore, the release of other anterior pituitary hormones in the presence of IL-1β was measured. Anterior pituitary cells from male Wistar rats were cultured for 3 days in medium containing 10% FCS. Incubations were performed at 37 C in medium with 0.5% BSA for measurement of $[^{125}I]T_3$ uptake and with 0.1% BSA for measurement of $[^{125}I]T_4$ uptake. Exposure to IL-1β (1 pM-1 nM) or TNFα (100 pM) for 2–4 h resulted in a significant decline in TSH release, which was almost 50% ($P < 0.05$) for 1 nM IL-1β and 24% ($P < 0.05$) for 100 pM TNFα. Measurement of other anterior pituitary hormones (FSH, LH, PRL, and ACTH) in the same incubation medium showed that IL-1β did not alter their release. When the effects of IL-1β (1 pM-1 nM) and TNFα (100 pM) on TRH-induced TSH release were measured in short term experiments, the inhibitory effects had disappeared. The addition of 1–100 nM octreotide, a somatostatin analog, resulted in a decrease in TRH-induced TSH release up to 33% of the control value ($P < 0.05$). Exposure to dexamethasone (1 nM to 1 μM) affected basal and TRH-induced TSH release similar to the effect of IL-1β. The 15-min uptake of $[^{125}I]T_3$ and $[^{125}I]T_4$, expressed as femtomoles per pM free hormone, was not affected by the presence of IL-1β (1–100 pM). When IL-1β (100 pM) was present during 3 days of culture, TSH release was reduced to 88 ± 2% of the control value ($P < 0.05$). This effect was not associated with an altered $[^{125}I]T_3$ uptake (15 min to 4 h) or with any change in nuclear T_3 binding. We conclude that 1) IL-1β decreases TSH release by a direct action on the pituitary; 2) this effect is not due to elevated thyroid hormone uptake or increased T_3 nuclear occupancy; 3) IL-1β does not affect TRH-induced TSH release or the release of other anterior pituitary hormones; and 4) TNFα affects basal and TRH-induced TSH release in the same way as IL-1β. (*Endocrinology* **137:** 1591–1598, 1996)

DURING SYSTEMIC illness, striking changes occur in serum thyroid parameters, a condition referred to as nonthyroidal illness (NTI). Most prominent are the low T_3 and elevated rT_3 together with a serum TSH level that is unaltered or even diminished (1, 2). Both the impaired metabolism of rT_3 and the reduced conversion of T_4 to T_3 are probably caused by a decrease in type I deiodinase activity (3). In addition, inhibition of transport of thyroid hormones into the liver contributes to the low serum T_3 level (4). Recently, a number of compounds that inhibit the transport of T_4 into peripheral tissues have been identified (5, 6). However, as these same compounds did not affect thyroid hormone transport into the pituitary (7), the mechanism preventing the serum TSH level from rising remains unknown.

In recent studies, a role for cytokines, especially interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNFα), in the pathogenesis of NTI was suggested (8–10). Cytokines are soluble peptides, mainly produced by macrophages and lymphocytes after immune challenge or inflammation, and are known for their regulatory role in the immune response. However, they are now also regarded as important factors that intimately link the immune and neuroendocrine systems (11). Attempts to prove that the decrease in TSH level in animals treated with cytokines was due to a central effect (*i.e.* diminished production of TRH) have not produced clear evidence (12, 13). Also, IL-1 receptors and IL-1 receptor messenger RNA were found in anterior pituitary cells of mice (14–16) and in the anterior pituitary tumor cell line AtT-20 (17, 18). Finally, a recent *in vitro* study with anterior pituitary cells reported an effect of TNFα on TSH release (19). Taken together, these findings suggest that cytokines, and in particular IL-1, could have a direct effect on the pituitary.

We, therefore, examined the effects of IL-1β and TNFα on TSH release of cultured anterior pituitary cells. Also, cellular T_3 and T_4 uptake, nuclear T_3 occupancy, and release of other anterior pituitary hormones in the presence of IL-1β were determined. This was performed to investigate whether IL-1β-induced changes in TSH release were related to altered thyroid hormone uptake and nuclear T_3 occupancy and whether IL-1β affected the thyrotropic cells specifically or the effect was the result of a more general action of IL-1β on the endocrine cells of the anterior pituitary. Furthermore, as both somatostatin and cortisol are important regulators of TSH secretion in response to stress or disease (20), we also tested the effects of octreotide (a somatostatin analog) and dexamethasone.

Serum concentrations of IL-1β in healthy subjects, in patients with sepsis or rheumatoid arthritis, and after endotoxin infusion were all in the picomolar range (21, 22).

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However, as local IL-1β production in the pituitary has been found (23), the actual tissue concentration may be higher than the measured serum values. Furthermore, the effects of IL-1β on pituitary hormone release *in vitro* were most prominent at concentrations ranging from 1 pM to 1 nM (24), whereas cytotoxicity was first observed at an IL-1β concentration of 10 nM (25). Therefore, we used IL-1β concentrations varying from 1 pM to 1 nM.

Materials and Methods

Materials

All solutions used for cell isolation and cell culture were obtained from Life Technologies Europe (Breda, The Netherlands), with the exception of human serum albumin (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), dispase (grade II; Boehringer, Mannheim, Germany), and fungizone (Bristol-Myers Squibb, Woerden, The Netherlands). Culture dishes (48 wells) were obtained from Costar (Cambridge, MA). Human recombinant IL-1β and human recombinant TNFα were purchased from Genzyme Corp. (Cambridge, MA). Octreotide was purchased from Sandoz (Basel, Switzerland), CRF from UCB (Brussels, Belgium), and TRH from Hoechst (Frankfurt am Main, Germany). Piperazine-*N,N'*-bis-[2-ethane sulfonic acid], HEPES, *N,N*-bis-[2-hydroxyethyl]2-aminoethane sulfonic acid, BSA (fraction V), dexamethasone, and *N*-nitro-D-arginine methyl ester (L-NAME) were obtained from Sigma Chemical Co. (St. Louis, MO). L-T_3 (T_3) was purchased from Henning Berlin (Berlin, Germany). [3',5'-^{125}I]T_4 (1500 μCi/μg) and [3'-^{125}I]T_3 (3070 μCi/μg) were obtained from Amersham International (Aylesbury, UK). Ingredients for the rat TSH RIA were kindly provided by the NIDDK (Bethesda, MD). Sephadex LH-20 and G-25 were obtained from Pharmacia (Uppsala, Sweden). All other reagents were of the highest purity available.

Animals

All experiments were performed using male Wistar rats, weighing 200–220 g. The animals had free access to food and water and were kept in a controlled environment (21 C) with constant day length (12 h).

Cell culture

Animals (n = 12–16 for each experiment) were killed between 0900–0930 h by decapitation. The pituitary glands were removed within 5 min, the neurointermediate lobe was discarded, and the anterior lobes were collected in calcium- and magnesium-free Hanks' Balanced Salt Solution supplemented with 10 g/liter human serum albumin, penicillin (10^5 U/liter), fungizone (0.5 mg/liter), and sodium bicarbonate (0.4 g/liter). Anterior pituitary cells were dissociated with dispase (final concentration, 2.4×10^3 U/liter), as described in detail previously (26). From each pituitary, 1.5×10^6 cells were obtained, and the viability of the cells, determined by trypan blue exclusion, was greater than 90%.

The cells were cultured at 37 C in a water-jacketed incubator with 5% CO_2 at a density of $4–8 \times 10^5$ cells/well in 48-well culture dishes. The cells were attached to the wells after 2 days of culture. On day 3, the cells were used for experiments. The culture medium consisted of MEM with Earle's salts supplemented with nonessential amino acids, sodium pyruvate (1 mmol/liter), 10% FCS, penicillin (10^5 U/liter), fungizone (0.5 mg/liter), L-glutamine (2 mmol/liter), and sodium bicarbonate (2.2 g/liter; pH 7.4) (26–28).

Hormone release and cellular content

The culture medium was removed and centrifuged ($2000 \times g$), and the supernatant was frozen until hormone determination. The incubation medium was identical to the culture medium, except that the FCS was replaced by 0.5% BSA. The effects of IL-1β and other compounds on basal release of TSH, LH, FSH, ACTH, and PRL were measured after 2–4 h of incubation (A), effects on TRH-stimulated TSH release were examined during 2-h exposure after 2 h of preincubation (B), and the possible role of nitric oxide (NO) in the effect of IL-1β on TSH release was examined in 4-h experiments by simultaneous incubation of IL-1β with the NO synthase inhibitor L-NAME (C) all at 37 C.

For A and C, cells were washed once with incubation medium and incubated for 2–4 h with TNFα (100 pM), octreotide (100 pM), dexamethasone (1 nM to 1 μM), IL-1β (1 pM to 1 nM) without or with L-NAME (0.05–0.5 mM), or TRH (100 nM). After incubation, the medium was removed, centrifuged ($2000 \times g$), and stored at −20 C.

For B, TRH-stimulated TSH release was examined after preincubation for 2 h without or with TNFα (100 pM), IL-1β (1 pM to 1 nM), dexamethasone (1 nM to 1 μM), or octreotide (1–100 nM). The preincubation medium was discarded, and incubation was continued for 2 h without or with TRH (100 nM) and the additions described above. The TSH response to TRH was used to ascertain the presence of active thyrotrophs in the preparation. As freshly isolated anterior pituitary cells were used for every experiment, and therefore, the quality of cells could vary between experiments, the magnitude of the TSH response to TRH was variable. However, in every experiment a significant increase in TSH release after the addition of TRH was observed. For determination of the cellular TSH content, 0.5 ml incubation medium was added to the wells. The cells were scraped from the wells with a rubber policeman, and the wells were sonicated twice for 30 sec each time. The extracts were removed, and the wells were washed with 0.5 ml medium. The two fractions were then combined (volume of cell extract, 1.0 ml) and centrifuged ($2000 \times g$) at room temperature, and the supernatant was frozen until further analysis.

Hormone determinations

TSH, LH, FSH, and PRL were determined by RIA as described previously (26–29). ACTH release from rat pituitary cells was estimated by an immunoradiometric assay for determination of human ACTH in plasma (CIS Biointernational, Gif-sur-Yvette, France).

Cellular [^{125}I]T_3 and [^{125}I]T_4 uptake

Cellular uptake experiments were performed with variable incubation times (15 min to 4 h). After removal of the culture medium, cells were preincubated with incubation medium. This medium was identical to the culture medium, except that the FCS was replaced by 0.5% BSA when uptake of [^{125}I]T_3 (50,000 cpm; 50 pM) was measured or with 0.1% BSA when uptake of [^{125}I]T_4 was determined (100,000 cpm; 175 pM). Preincubation (0.25 ml) was carried out for 30 min at 37 C in the presence or absence of various concentrations of IL-1β (1–100 pM). Incubation started by adding 10 μl [^{125}I]T_3 or [^{125}I]T_4 directly to the preincubation medium and lasted 15 min. In the time-course experiments, IL-1β (100 pM) was added to the culture medium. The experiments were performed as described above, except that the preincubation medium did not contain any additions and was discarded after 30 min. Thereafter, 0.25 ml incubation medium containing [^{125}I]T_3 was added to the cells for 15 min, 1 h, or 4 h.

Incubations lasting for more than 1 h were performed at 37 C in humidified air with 5% CO_2. Incubations of shorter duration took place in a 37 C incubation chamber on a rotating device without CO_2. Therefore, the $NaHCO_3$ in the incubation medium was replaced by an equimolar amount of HEPES (8.9 mM), piperazine-*N,N'*-bis-[2-ethane sulfonic acid] (10.6 mM), and *N,N*-bis-[2-hydroxyethyl]2-aminoethane sulfonic acid (11.2 mM).

After incubation, the medium was removed, and the cells were washed once with 1 ml ice-cold saline to remove the tracer not bound to the cells. Cells were dissolved in 1 ml 0.1 N NaOH and were counted for ^{125}I activity in a 16-channel γ-counter (NE 1600, Nuclear Enterprises, Edinburgh, Scotland). The amount of [^{125}I]T_3 or [^{125}I]T_4 taken up by the cells was expressed as a percentage of the added radioactivity (percent dose). The procedure described above was also applied to incubations without cells (blanks). All results were corrected for the radioactivity observed in the blanks.

Nuclear binding of [^{125}I]T_3

After incubation, cells were washed with 1 ml ice-cold saline (0.9% NaCl). All of the following actions were performed on ice. Cells were

scraped from the wells with a rubber policeman in 1 ml PBS and counted for 1 min. Then, they were centrifuged (300 × *g*; 4 C; 7 min) and counted (1 min) while 1 ml PBS-0.5% Triton X-100 was added to the cell pellet, as previously described (30). Cells were vortexed for 2 min and centrifuged (900 × *g*; 4 C; 5 min) to reveal the nuclei. The nuclear pellet was counted for 5 min.

DNA determination

The DNA content of cells or nuclear pellets was determined using a modification (31) of the fluorescence technique described by Downs and Wilfinger (32).

Free hormone fraction

Calculation of the free T_3 (FT_3) or free T_4 (FT_4) concentration was based on determination of the free fractions by equilibrium dialysis (33). In medium containing 0.5% BSA, the FT_3 fraction was 3.78 ± 0.15% (n = 4), whereas the FT_4 fraction was 3.60 ± 0.17% (n = 4) in medium containing 0.1% BSA. The addition of variable concentrations of IL-1β (1 and 100 pM) altered the free fractions of both iodothyronines only slightly, to 3.55 ± 0.02% (n = 4) and 3.50 ± 0.04% (n = 4) for the FT_3 fraction and to 3.76 ± 0.37% (n = 4) and 3.58 ± 0.45% (n = 4) for the FT_4 fraction. Nevertheless, the net cellular uptake of radiolabeled T_3 and T_4 was corrected for these slight changes in free hormone concentrations by expressing the data in femtomoles per pM free hormone.

Statistics

The statistical significance of the effects of the various compounds tested on TSH, LH, FSH, PRL, and ACTH release; the uptake of [^{125}I]T_3 and [^{125}I]T_4; and the nuclear binding of radiolabeled T_3 was calculated by Student's *t* test for unpaired observations or Duncan's test for a repeated measure design. $P < 0.05$ was regarded as statistically significant.

Results

Short term effects of cytokines, dexamethasone, and octreotide

Basal TSH release. To determine the effects of IL-1β, TNFα, dexamethasone, or octreotide on basal TSH release, these compounds were added during 2- to 4-h incubations. Addition of IL-1β (1 pM-1 nM) for 4 h resulted in a dose-dependent decrease in TSH release; the highest concentration produced a maximum inhibitory effect of almost 50% ($P < 0.05$). The cellular TSH content showed no significant change, nor did the total TSH, expressed as the sum of medium and cells (Table 1). This indicated that IL-1β was not toxic to the cells, which was also confirmed by measurement of the cellular DNA content after 4 h of incubation. The DNA content (micrograms per well) was 1.64 ± 0.03 (n = 3) in the presence of 1 nM IL-1β and 1.51 ± 0.05 (n = 6) in control cells. Exposure to 100 nM TRH resulted in a significant rise (38%; $P < 0.05$) in TSH release, without a significant change in the cellular or total TSH content. The addition of another major cytokine, TNFα, at a concentration of 100 pM reduced basal TSH release by 24 ± 5% (n = 9; $P < 0.05$).

In two experiments, the effects of dexamethasone (1 nM to 1 μM) and octreotide (100 nM) were examined after 2 h of incubation. Addition of 1 nM dexamethasone resulted in a decline in TSH release of approximately 20% ($P < 0.05$), an effect similar to that observed at the highest dexamethasone concentration of 1 μM (24%; $P < 0.05$; Fig. 1). Octreotide, however, did not affect basal TSH release at the concentration tested (not shown).

TRH-induced TSH release. In two experiments, the same compounds as those used above were tested for their effects on TRH-induced TSH release during short (2-h) exposure. Data were expressed as a percentage of the control values (TRH alone). Figure 2A shows that IL-1β had no effect on TRH-stimulated TSH release at concentrations varying from 1 pM to 1 nM. TNFα at a concentration of 100 pM also did not alter TRH-induced TSH release (n = 6; not shown). The stimulation of TSH release by TRH in these experiments was more than 400% (not shown), indicating that the anterior pituitary cells responded well to TRH. Dexamethasone did not affect TRH-stimulated TSH release at the concentrations tested (10 nM to 1 μM; Fig. 2B). However, octreotide was a very potent inhibitor of the stimulatory effect of TRH on TSH release at any concentration tested; the lowest (1 nM) produced a maximum inhibition of 67% ($P < 0.05$; Fig. 2C).

Release of other pituitary hormones. In the incubation medium from two of the experiments shown in Table 1, FSH and LH were also determined (Table 2). Cells were incubated for 4 h without or with IL-1β in concentrations ranging from 1–100 pM. Addition of the lowest concentration showed a significant decrease in hormone release for both FSH and LH ($P < 0.05$). However, at higher concentrations this inhibitory effect of IL-1β disappeared, and hormone release was not different from the control values. Incubation with TRH did not affect FSH or LH release.

The effect of IL-1β (1–100 pM) on ACTH release was de-

TABLE 1. Effects of variable concentrations of IL-1β on TSH release and cellular content of anterior pituitary cells and total TSH as the sum of TSH content in medium and cells

Exp conditions	TSH content (ng)		
	Medium	Cells	Total
No additions (12)	6.37 ± 0.46	34.88 ± 1.89	41.25 ± 1.98
+ 1 pM IL-1β (12)	4.58 ± 0.33[a]	34.10 ± 1.88	38.68 ± 1.91
+ 10 pM IL-1β (12)	4.86 ± 0.28[a]	32.63 ± 2.30	37.49 ± 2.40
+ 100 pM IL-1β (12)	4.33 ± 0.13[a]	31.42 ± 2.48	35.75 ± 2.47
+ 1 nM IL-1β (6)	3.40 ± 0.14[a]	32.49 ± 1.25	35.89 ± 1.32
+ 100 nM TRH (10)	8.81 ± 0.42[a]	28.21 ± 1.68	37.02 ± 1.87

Data show the mean of 6–12 observations in 2–4 experiments. Anterior pituitary cells (400,000 cells/well) were cultured for 3 days in medium containing 10% FCS. After removal of the culture medium, cells were incubated for 4 h without or with addition of IL-1β or TRH at the concentrations shown above. TSH was measured in medium and cells by RIA. Also, the total TSH content was calculated as the sum of medium and cells.

[a] $P < 0.05$ *vs.* no additions.

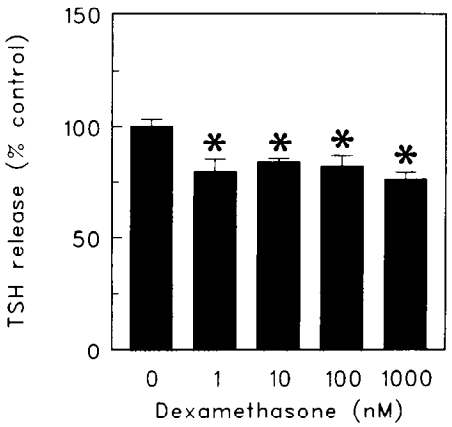

FIG. 1 The effect of dexamethasone on basal TSH release. Anterior pituitary cells were cultured for 3 days at a density of 400,000 cells/well. Cells were preincubated for 2 h in incubation medium containing 0.5% BSA. This was followed by incubation for 2 h in medium without or with variable concentrations of dexamethasone. TSH released in the presence of dexamethasone is expressed as a percentage of the control value, *i.e.* TSH release in the absence of any additions. *Bars* show the mean ± SE of three to six observations from two experiments with control values of 5.7 ± 0.1 (n = 3) and 9.6 ± 0.6 (n = 3) ng TSH/well respectively. *, $P < 0.05$ *vs.* no additions.

termined after 4 h of incubation and compared with that of CRF (10 nM). The addition of CRF resulted in a 5-fold stimulation of ACTH release, from 4 ± 1 ng/ml (n = 3) to 18 ± 1 ng/ml (n = 4) ($P < 0.05$). The presence of IL-1β did not change ACTH release at any of the concentrations tested (not shown). The addition of CRF did not affect TSH release (not shown), whereas the presence of 100 pM IL-1β resulted in a decline in TSH release of approximately 25% ($P < 0.05$), *i.e.* an effect similar to that described above.

Finally, the incubation medium of one of the experiments presented in Table 1 was used to measure PRL levels. PRL release in the absence of any addition was 1200 ± 50 ng/ml (n = 3), and the presence of IL-1β did not alter PRL release, which was 1260 ± 60, 1300 ± 50, 1330 ± 40, and 1300 ± 270 ng/ml with 1, 10, 100, and 1000 pM IL-1β, respectively (n = 3 for all).

Initial uptake of [^{125}I]T_3 and [^{125}I]T_4. A series of experiments was performed to study the effect of IL-1β on the initial uptake of [^{125}I]T_3 and [^{125}I]T_4 at the level of the pituitary plasma membrane, *i.e.* after 15 min of incubation. [^{125}I]T_3 uptake was measured in medium containing 0.5% BSA, whereas [^{125}I]T_4 uptake was determined in medium with 0.1% BSA. This was performed to increase the free hormone fraction of T_4 and, thus, the availability of T_4 to the pituitary cells (34).

The 15-min uptakes of [^{125}I]T_3 and [^{125}I]T_4 expressed per pM free hormone were 0.101 ± 0.008 (n = 20) and 0.027 ± 0.003 (n = 15), respectively, in good agreement with previous results obtained with the same cell preparation (34, 35). Neither [^{125}I]T_3 uptake nor that of [^{125}I]T_4 was affected by IL-1β in the concentration range 1–100 pM (Fig. 3).

Long term effects of IL-1β on basal TSH release and [^{125}I]T_3 uptake

TSH release. To examine the effect of long term exposure to IL-1β, pituitary cells were cultured in the presence of 100 pM

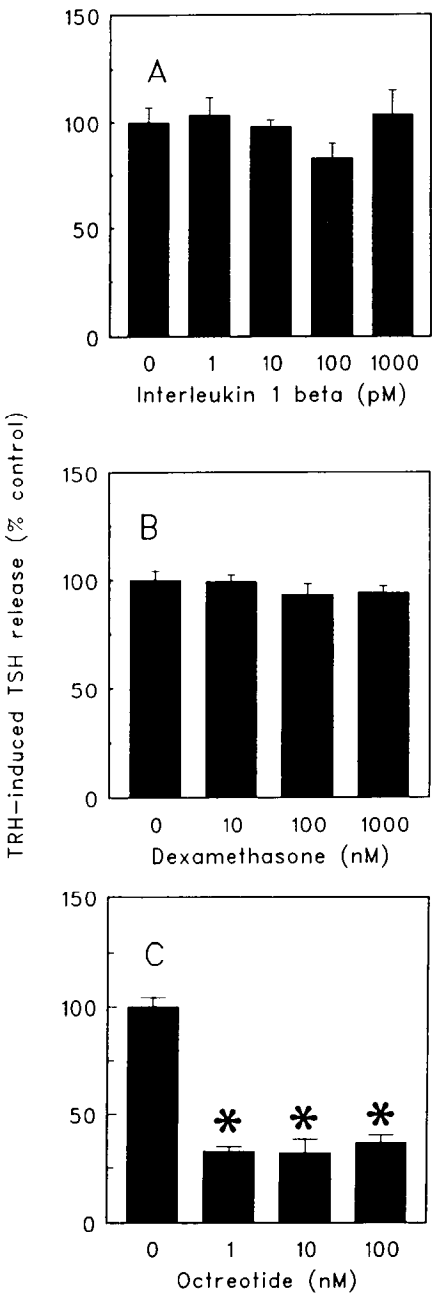

FIG. 2 Effects of IL-1β (A), dexamethasone (B), and octreotide (C) on TRH-induced TSH release. Anterior pituitary cells were cultured for 3 days at a density of 400,000 cells/well. Thereafter, cells were preincubated for 2 h in incubation medium in the presence or absence of variable concentrations of IL-1β (A), dexamethasone (B), or octreotide (C). Incubations were performed by adding TRH (100 nM) alone or together with the compounds mentioned above to the cells and lasted 2 h. TSH released in the presence of TRH and variable concentrations of IL-1β, dexamethasone, or octreotide was expressed as a percentage of the TSH released in the presence of TRH alone. *Bars* represent the mean ± SE of three to six observations from two experiments, with basal TSH values of 3.2 ± 0.1 (n = 2) and 7.8 ± 1.0 (n = 3) ng/well. *, $P < 0.05$ *vs.* controls.

IL-1β, which was added from day 0. As in the short term experiments, the effect of IL-1β was compared with those of TRH (1 nM) and octreotide (1 nM). Figure 4 shows the effects of these compounds on TSH release. Long term exposure to

TABLE 2. Effect of 4-h incubation with variable concentrations of IL-1β on FSH and LH release from anterior pituitary cells

Exp conditions	Hormone release (ng)	
	FSH	LH
No additions (6)	5.99 ± 0.58	8.59 ± 1.69
1 pM IL-1β (6)	4.49 ± 0.31[a]	5.45 ± 0.59[a]
10 pM IL-1β (6)	5.49 ± 0.56	6.53 ± 0.67
50 pM IL-1β (6)	5.01 ± 0.36	6.70 ± 0.73
100 pM IL-1β (6)	4.90 ± 0.22	8.23 ± 0.58
100 nM TRH (6)	4.97 ± 0.17	7.62 ± 0.68

Data show the mean ± SE of six observations in two experiments. Anterior pituitary cells were cultured for 3 days in medium containing 10% FCS. Then culture medium was removed, and cells were incubated for 4 h in medium without or with variable concentrations of IL-1β. Thereafter, FSH and LH were measured in medium by RIA.
[a] $P < 0.05$ *vs.* no additions.

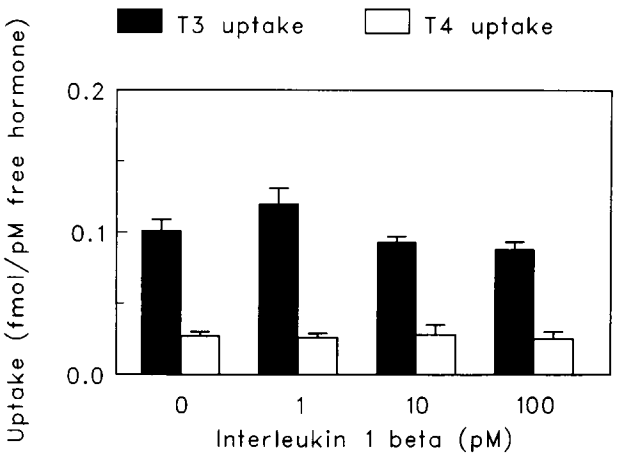

FIG. 3 Uptake of $[^{125}I]T_3$ and $[^{125}I]T_4$ by male anterior pituitary cells in the presence of IL-1β. Cells were cultured for 3 days at a density of 500,000 cells/well. Thereafter, culture medium was removed, and cells were washed once with incubation medium containing 0.5% BSA when $[^{125}I]T_3$ was measured and 0.1% BSA in the case of $[^{125}I]T_4$. Then, cells were preincubated for 30 min in incubation medium without or with various concentrations of IL-1β, whereas incubation started by adding 10 μl $[^{125}I]T_3$ (50,000 cpm/well) or $[^{125}I]T_4$ (100,000 cpm/well) directly to the preincubation medium. Uptake of both tracers, measured for 15 min, is expressed per pM free hormone. *Bars* represent the mean ± SE of 9–20 observations from 3–7 experiments. None of the differences in $[^{125}I]T_3$ uptake was statistically significant.

IL-1β reduced TSH release of the pituitary cells to 88 ± 2% ($P < 0.05$) of the control value (Fig. 4, *second column*). Addition of TRH stimulated TSH release by 27 ± 7% ($P < 0.05$), indicating the presence of active thyrotropic cells. Octreotide reduced TSH release to 91 ± 3% ($P < 0.05$) of the control value (Fig. 4, *last column*).

$[^{125}I]T_3$ uptake. To examine the effect of long term exposure to IL-1β on the cellular uptake of T_3, anterior pituitary cells were cultured without or with 100 pM IL-1β. The addition of 100 pM IL-1β resulted in a decrease in TSH release of 15% compared with the control value (n = 15; $P < 0.05$). The time course of $[^{125}I]T_3$ uptake was measured at 15 min, 1 h, and 4 h of incubation (Fig. 5). The uptake of radioactive T_3 in pituitary cells cultured in the presence or absence of IL-1β showed a steep rise up to 1 h of incubation and then leveled off. In contrast with the effect on TSH release, IL-1β did not affect $[^{125}I]T_3$ uptake at any time measured (Fig. 5).

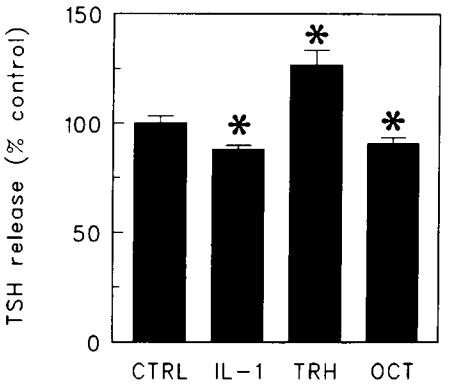

FIG. 4 Effect of long term exposure to IL-1β (100 pM), TRH (1 nM), and octreotide (OCT; 1 nM) on TSH release by anterior pituitary cells (800,000 cells/well). Compounds were added on day 0 to the culture medium. After 3 days of culture, the medium was removed, and TSH release was measured. Data represent the mean of 12–17 observations from 2 independent experiments and are expressed as a percentage of the control value, *i.e.* cells cultured in the absence of any additions. Absolute control values were 99.5 ± 1.6 (n = 9) and 103.5 ± 7.1 (n = 8) ng TSH/well, respectively. *, $P < 0.05$ *vs.* no additions.

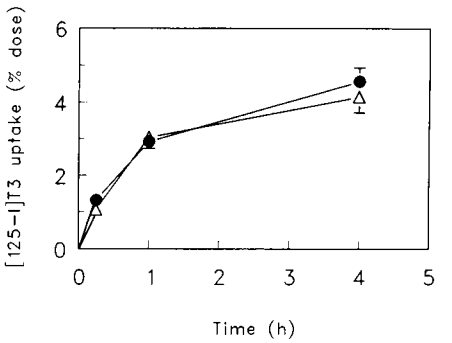

FIG. 5 Effect of long term exposure to IL-1β on the cellular uptake of $[^{125}I]T_3$. Rat anterior pituitary cells were cultured for 3 days in presence (△) or absence (●) of 100 pM IL-1β. After 3 days, culture medium was removed, and cells were preincubated for 30 min in incubation medium containing 0.5% BSA. This was followed by incubation in the same medium with $[^{125}I]T_3$ (50,000 cpm/well) for 15 min, 1 h, and 4 h. Uptake is expressed as a percentage of the total added radioactivity (percent dose). Data represent the mean ± SE of five or six observations from two independent experiments.

Nuclear binding of $[^{125}I]T_3$. We also examined whether IL-1β influenced nuclear binding of $[^{125}I]T_3$. Anterior pituitary cells (800,000 cells/well) were cultured for 3 days in the presence or absence of 100 pM IL-1β. Thereafter, cells were incubated for 2 h with $[^{125}I]T_3$ in the absence or presence of 10 μM T_3.

In Table 3, the radioactivity detected in cellular and nuclear pellets is shown. Again, IL-1β did not change cellular uptake or alter nuclear binding of $[^{125}I]T_3$. The addition of 10 μM T_3 during incubation decreased cellular $[^{125}I]T_3$ uptake by approximately 55%, whereas nuclear uptake of the tracer was completely inhibited. The *last column* of Table 3 shows the DNA content of the nuclear pellets, which was similar for the three culture/incubation conditions. When nuclear bound $[^{125}I]T_3$ was expressed per μg DNA, the value for the control incubations was 0.42 ± 0.04 (n = 4), and for the cells cultured in presence of IL-1β, the value was 0.24 ± 0.01 (n = 3; $P < 0.05$).

TABLE 3. Effects of presence of IL-1β during culture on cellular and nuclear bound [^{125}I]T_3 and DNA content in cultured anterior pituitary cells

Culture conditions	Exp conditions	[^{125}I]T_3 radioactivity (% dose)		DNA content nuclei (μg)
		Cell pellet	Nuclear pellet	
No additions (4)	No additions	2.17 ± 0.12	0.47 ± 0.08	1.11 ± 0.08
No additions (4)	+ 10 μM T_3	0.95 ± 0.09[a]	0.03 ± 0.01[a]	1.19 ± 0.13
+ 100 pM IL-1β (3)	No additions	2.20 ± 0.10	0.32 ± 0.01	1.34 ± 0.09

Data show the mean ± SE of three or four observations in a single experiment. Anterior pituitary cells were cultured for 3 days at a density of 800,000 cells/well. IL-1β was added on day 0 to the culture medium. After preincubation for 30 min, cells were incubated for 2 h in medium containing 0.5% BSA and [^{125}I]T_3 (100,000 cpm) in the absence or presence of 10 μM unlabeled T_3. Cells were scraped from the wells with 2 × 0.5 ml ml ice-cold PBS. After centrifugation, the cell pellet was counted. The nuclear pellet was obtained after the addition of Triton X-100, followed by centrifugation. For further details, see *Materials and Methods*.

[a] $P < 0.05$ *vs.* no additions.

Role of NO in the effect of IL-1β on TSH release

In two experiments we investigated the role of NO in the effect of IL-1β on basal TSH release. Anterior pituitary cells were incubated for 4 h with 100 pM IL-1β alone or together with the NO synthase inhibitor L-NAME at concentrations of 0.05 and 0.5 mM. The inhibitory effect of 100 pM IL-1β on TSH release (38%) was similar to that observed in our previous experiments, but this effect was not reversed by L-NAME at the concentrations tested (Table 4).

TABLE 4. Effect of IL-1β alone or combined with L-NAME on TSH release during 4-h exposure

Exp conditions	TSH release (ng)	Effect (%)
No additions (17)	11.06 ± 0.41	
100 pM IL-1β (17)	6.81 ± 0.47[a]	−38
100 pM IL-1β + 0.05 mM L-NAME (12)	6.92 ± 0.36[a]	−37
100 pM IL-1β + 0.5 mM L-NAME (12)	7.15 ± 0.53[a]	−35
100 nM TRH (9)	16.15 ± 0.43[a]	+46

Data show the mean ± SE of 9–17 observations from 2 experiments. Anterior pituitary cells (600,000 cells/well) were cultured for 3 days in medium containing 10% FCS. Then, culture medium was removed, and cells were incubated for 4 h in medium without or with IL-1β (100 pM) in the absence or presence of the NO synthase inhibitor L-NAME (0.05 and 0.5 mM). TSH was measured by RIA.

[a] $P < 0.05$ *vs.* no additions.

Discussion

The present study performed with cultured rat anterior pituitary cells shows that IL-1β and TNFα had an inhibitory effect on TSH release during both short and long term exposures, whereas this effect was not observed when IL-1β and TNFα were added together with 100 nM TRH. The decrease in TSH release could not be explained by elevated cellular T_3 or T_4 uptake or by increased nuclear binding of T_3 in the presence of IL-1β. Furthermore, the effect of IL-1β was specific for the thyrotropic cells, as the release of other pituitary hormones was not affected.

An inhibitory effect of cytokines on TSH release from cultured anterior pituitary cells was previously described (19). On the other hand, other studies with similar cell preparations reported an increase (24) or no effect (36). The reasons for these discrepancies are not clear. However, a reduction of TSH release by cytokines *in vitro* seems to be more consistent with the changes observed in thyroid parameters after cytokine administration *in vivo* (8–10).

Exposure to IL-1β resulted in a decrease in TSH release, which could not be explained by an increased intracellular T_3 concentration in the pituitary. Also, *in vivo* observations suggested that the effects of IL-1β were independent of thyroid hormone uptake, as administration of IL-1β to hypothyroid rats resulted in a decrease in serum TSH (37).

A question that should be addressed is whether the effect on TSH release is due to the exogenously added IL-1β or to endogenous compounds such as cytokines produced in the pituitary after exposure to IL-1β. Local production of IL-1β (23) and IL-6 (38, 39) in the pituitary has been demonstrated. The same studies showed that IL-1β is able to induce the production of both IL-1 and IL-6. Therefore, it is not excluded that the effect of IL-1β observed in our study derives from locally produced IL-1β or IL-6 triggered by the exogenous IL-1β. The possibility of a role for IL-6 as a mediator of the inhibitory effect of IL-1β on TSH release has been suggested by two *in vivo* studies in which administration of IL-6 diminished serum TSH levels (10, 12). Furthermore, the facts that serum IL-1 levels are low and only slightly elevated in severe states of illness (21, 22) and that cytokines exert their effects mainly locally favor the concept of locally produced cytokines affecting TSH release.

In a previous *in vivo* study (12), infusion of rats with IL-1α resulted in greater inhibitory effects on plasma thyroid hormone and TSH levels than those produced by IL-6. Furthermore, infusion of IL-1α, but not IL-6, resulted in an increase in serum corticosterone. The question was raised whether the difference in the effects of IL-1α and IL-6 on TSH serum levels could be explained by an additional effect of corticosterone on the thyrotropic cells. In our *in vitro* study, we found an inhibitory effect of dexamethasone on basal TSH release. Dexamethasone was previously found to reduce GH release by cultured anterior pituitary cells at similar concentrations (40). Octreotide, a somatostatin analog, also reduced basal TSH release during long term exposure, an effect that has been described previously (41, 42).

Although IL-1β affected basal TSH release, this effect disappeared in the presence of excess amounts of TRH. This could mean that excess TRH overruled the effect of IL-1β or that TRH-induced TSH release is regulated differently from basal TSH release. Furthermore, it is not excluded that there

is an effect of IL-1β on TRH-induced TSH release at submaximal concentrations of TRH. On the other hand, TNFα was recently reported to stimulate TSH release at submaximal concentrations of TRH (19). For dexamethasone, the same effect as that for IL-1β was observed, whereas exposure to octreotide reduced TRH-induced TSH release by 70%, an effect that has been observed in previous *in vitro* studies (43–45). The lack of IL-1β to affect TRH-induced TSH release together with the inhibitory effect of octreotide and the observation that IL-1β can stimulate the release of hypothalamic somatostatin (46) suggest an additional suprahypophysial action of IL-1β *in vivo*.

Furthermore, to exclude the possibility that the effect of IL-1β on basal TSH release was due to a general action on the pituitary, we measured the release of other pituitary hormones in the presence of IL-1β. IL-1β did not alter ACTH release, whereas CRF significantly enhanced it. In previous studies using comparable concentrations of IL-1β, conflicting results on ACTH release were reported, with both a stimulatory effect (24, 47) and no effect (48–50) found. Also, we did not find any effect of the higher IL-1β concentrations (10–100 pM) on the release of PRL, FSH, or LH, and this was in agreement with previous studies (47, 50). However, our study showed an as yet unexplained decrease in FSH and LH release at the lowest IL-1β concentration (1 pM).

Evidence exists that there is a functional link between IL-1 and NO, the product of oxidation of L-arginine to L-citrulline catalyzed by NO synthetase (51, 52). NO synthase activity has been observed in the cells of the anterior pituitary tumor cell line AtT-20/D16 (53), in the posterior pituitary of rats, and in nuclei of rat hypothalami closely associated with the regulation of pituitary activity (54). Therefore, a role for NO as a mediator of IL-1 action in the neuroendocrine system has been suggested. However, in our experiments this seemed to be of minor importance, because the effects of IL-1β on TSH release could not be reversed by the NO synthase inhibitor L-NAME.

Another possible mediator of the effect of IL-1β could be nerve growth factor (NGF). NGF immunoreactivity and NGF receptors were recently detected in thyrotropic and other anterior pituitary cells (55). Furthermore, IL-1β (1 nM) was able to stimulate NGF secretion (25). Together, these findings suggest that thyrotrophic cells can be the target of NGF action and that the effect of IL-1β can be mediated by locally produced NGF, which acts in an auto- or paracrine fashion on TSH release.

The present study shows an inhibitory effect of IL-1β on TSH release *in vitro* that is in agreement with the decrease in the serum TSH level observed after the administration of IL-1β *in vivo*. Thus, our findings support the postulated role of IL-1 as a mediator of the changes observed in serum thyroid hormone parameters during NTI. From our study it can be concluded that the decrease in TSH release can be explained by a direct effect of IL-1β on the thyrotrophic cells. The decrease in TSH release is not due to elevated thyroid hormone uptake, increased T_3 nuclear occupancy, or production of NO. Thus, the mechanism by which IL-1β exerts this effect remains to be established.

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