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Different Effects of Continuous Infusion of Interleukin-1 and Interleukin-6 on the Hypothalamic-Hypophysial-Thyroid Axis*

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ABSTRACT

The cytokines interleukin-1 (IL-1) and IL-6 are thought to be important mediators in the suppression of thyroid function during nonthyroidal illness. In this study we compared the effects of IL-1 and IL-6 infusion on the hypothalamus-pituitary-thyroid axis in rats. Cytokines were administered by continuous ip infusion of 4 μg IL-1α/day for 1, 2, or 7 days or of 15 μg IL-6/day for 7 days. Body weight and temperature, food and water intake, and plasma TSH, T_4, free T_4 (FT_4), T_3, and corticosterone levels were measured daily, and hypothalamic pro-TRH messenger RNA (mRNA) and hypophysial TSHβ mRNA were determined after termination of the experiments. Compared with saline-treated controls, infusion of IL-1, but not of IL-6, produced a transient decrease in food and water intake, a transient increase in body temperature, and a prolonged decrease in body weight. Both cytokines caused transient decreases in plasma TSH and T_4, which were greater and more prolonged with IL-1 than with IL-6, whereas they effected similar transient increases in the plasma FT_4 fraction. Infusion with IL-1, but not IL-6, also induced transient decreases in plasma FT_4 and T_3 and a transient increase in plasma corticosterone. Hypothalamic pro-TRH mRNA was significantly decreased (−73%) after 7 days, but not after 1 or 2 days, of IL-1 infusion and was unaffected by IL-6 infusion. Hypophysial TSHβ mRNA was significantly decreased after 2 (−62%) and 7 (−62%) days, but not after 1 day, of IL-1 infusion and was unaffected by IL-6 infusion. These results are in agreement with previous findings that IL-1, more so than IL-6, directly inhibits thyroid hormone production. They also indicate that IL-1 and IL-6 both decrease plasma T_4 binding. Furthermore, both cytokines induce an acute and dramatic decrease in plasma TSH before (IL-1) or even without (IL-6) a decrease in hypothalamic pro-TRH mRNA or hypophysial TSHβ mRNA, suggesting that the acute decrease in TSH secretion is not caused by decreased pro-TRH and TSHβ gene expression. The TSH-suppressive effect of IL-6, either administered as such or induced by IL-1 infusion, may be due to a direct effect on the thyrotroph, whereas additional effects of IL-1 may involve changes in the hypothalamic release of somatostatin or TRH. As glucucorticoids are known to suppress hypothalamic TRH mRNA levels, it is speculated that the decrease in pro-TRH gene expression caused by prolonged infusion of IL-1 is mediated by the high plasma corticosterone levels. (*Endocrinology* **135:** 1336–1345, 1994)

DURING acute and chronic systemic illness, profound changes in thyroid function occur in both humans (1–3) and animals (4). In humans, the most characteristic changes are a decrease in the plasma T_3 level and an increase in the plasma level of rT_3. Plasma T_4 may also be decreased in severely ill patients (3), mainly due to reduced binding to transport proteins (5, 6), as plasma free T_4 (FT_4) usually remains within the normal range. It has been suggested that cytokines are important mediators of the changes in thyroid economy during diseases in which the immune system is activated (4, 7–11). Cytokines are polypeptides primarily produced by activated monocytes and macrophages, which play important roles not only in regulating the immune system, but also in interacting with several endocrine systems (12–17). In rats, a single injection of interleukin-1 (IL-1) lowered plasma TSH and thyroid hormone levels within 5 h (4). Continuous infusion of IL-1β induced in the rat decreases in plasma TSH, FT_4, and T_4 binding (18). It is, however, not fully understood how cytokines suppress the pituitary-thyroid function.

Inflammation stimulates the production of a cascade of cytokines, of which, in particular, tumor necrosis factor-α, IL-1, and IL-6 represent key factors for communication between the immune and neuroendocrine systems (19–21). As part of the pleiotropic effects of IL-1 is mediated by IL-6, we compared the effects of short and long term infusion of IL-1 and long-term infusion of IL-6 on the hypothalamic-pituitary-thyroid axis. To identify the sites of action of IL-1 and IL-6, their effects were measured on plasma T_4, FT_4, T_3, TSH, and corticosterone; TRH content in median eminence; hypothalamic levels of pro-TRH messenger RNA (mRNA); and pituitary levels of TSHβ mRNA. As hepatic type I deiodinase is responsible for 60–70% of peripheral T_3 production in euthyroid rats (22), we also measured the activity of this enzyme during IL infusion.

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Materials and Methods

Materials

Recombinant human IL-1α (IL-1) was kindly provided by Dr. P. Lomedico (Hoffman LaRoche, Nutley, NJ). The preparation, supplied in 50 mM potassium phosphate (pH 6.5) and 0.1 M sodium chloride, had an activity of 2×10^8 U/ml (D10 assay) and a specific activity of 3×10^8 U/mg protein. According to the specifications of the suppliers, endotoxin contamination was negligible (0.5 U/ml IL-1 solution, as detected in the limulus amoebocyte lysate assay).

Human IL-6, produced by recombinant DNA technology in *Escherichia coli*, was obtained from Sandoz (Sandoz Forschungsinstitut, Vienna, Austria). The specific activity of the preparation was 52×10^6 U/mg (by B13.29 assay). The preparation (SDZ 280–969, batch PPG9001) was supplied in 20 mM sodium phosphate (pH 6.7), and endotoxin contamination was negligible (<0.4 U/mg protein).

Both IL-1 and IL-6 were diluted in sterile pyrogen-free saline [0.9% NaCl (wt/vol) in water]. All chemicals used were of analytical grade. The concentrations of IL-1 and IL-6 used for infusion in this study were based upon the findings of a previous study performed by Hermus *et al.* (18) and a pilot study in which three concentrations of IL-6 infusion were studied in rats (data not shown).

Animals

Male albino Wistar rats (Cpb:WU) were obtained from the local breeding facility and individually housed in Plexiglass cages in an artificially lighted room (lights on at 0700 h; lights off at 1900 h). Rats were provided with commercial rat chow containing 22% protein, 4.8% fat, and 66.8% carbohydrates (RMH-TH, Hope Farms, Woerden, The Netherlands) and tap water *ad libitum*. At the time of the start of the experiments, rats were 10 weeks old and weighed 200–220 g. Animal procedures were approved by the institutional review board.

Experimental design

Long term infusion. To diminish the stress of the experimental procedure, rats were handled daily, starting at least 1 week before the insertion of an indwelling cannula into an external jugular vein. Rats were cannulated according to the method described by Steffens (23) with some minor modifications (24). After insertion, the cannula was filled with a 0.9% NaCl solution containing heparin (500 IU/ml; Organon Teknika, Boxtel, The Netherlands) and polyvinylpyrrolidone (1 g/ml; Merck, Darmstadt, Germany).

Seven to 9 days after cannulation, rats were implanted with an Alzet osmotic minipump (model 2001, Alzet Corp., Palo Alto, CA; 1 μl/h for 7 days). Rats were infused for 7 days with IL-1 (4 μg/day) or IL-6 (15 μg/day) dissolved in sterile pyrogen-free physiological saline or with saline alone. The pumps were equilibrated by immersion in physiological saline solution for 3–4 h at 37 C according to the instructions of the manufacturer and then implanted ip in ether-anesthetized animals between 1400–1600 h (day 0). The indwelling cannula and the osmotic pump were tolerated well by the rats with no obvious signs of discomfort or infection.

From the freely moving rats, blood samples of 2 ml were withdrawn from the jugular venous cannula on several days of the experiment starting 2 days before implantation of the osmotic minipumps (18). Because of the circadian rhythm in hormone release, blood was sampled at about the same time each day (between 1000–1200 h). Blood samples were collected in prechilled tubes containing 60 μl 10% (wt/vol) EDTA in saline, gently shaken, and centrifuged for 10 min at $1500 \times g$ at 4 C. After removal of the plasma, the residue containing red blood cells was resuspended in sterile physiological saline solution (1.5 ml) and returned through the jugular venous cannula to each rat. Plasma samples were aliquoted and stored at −20 C until assayed.

In all rats, body weight was measured daily between 0815–0900 h. Body temperature was measured daily between 0815–0900 h and between 1300–1430 h in conscious hand-held rats by insertion of a thermal probe into the rectum. The probe was connected to a digital temperature monitor (Digital DT100, Elbatron, Kerkdriel, The Netherlands). Mean daily temperature for each rat was determined by averaging the morning and afternoon rectal temperatures. The daily food and water intake was estimated by weighing the residual food pellets and water for individual cages.

At the end of the experiment (day 7), the rats were killed by decapitation. The livers were cut into pieces, frozen in liquid nitrogen, and kept at −80 C until the estimation of type I deiodinase activity. The skull was opened, and the brain was removed. The hypothalamus was isolated (limits, posterior border of the chiasmatic opticum, anterior border of the mamillary bodies, and lateral hypothalamic border; height, ~3 mm) for the determination of pro-TRH mRNA. Also, the pituitary gland was isolated to estimate the level of TSHβ mRNA. Both tissues were snap-frozen in liquid nitrogen and kept at −80 C until determination of pro-TRH and TSHβ mRNAs.

Short term infusion. Rats were infused with IL-1 (4 μg/day) for 1 day (osmotic minipump model 2001D; 8 μl/h for 1 day) or 2 days (osmotic minipump model 1003D; 1 μl/h for 3 days) or with saline. The pumps were implanted between 1400–1600 h. These animals had not been implanted with a cannula into the jugular vein. After 1 or 2 days of infusion, trunk blood was collected after decapitation of the rats between 1200–1500 h. The livers, hypothalami, and pituitaries were collected according to the methods described above. From animals infused for 1 day with IL-1 or saline, the median eminence was also collected. This was performed by grasping the hypophysial stalk with forceps and lifting it from the brain. The protruding tissue fragment, comprising the hypophysial stalk and the median eminence, but referred to as median eminence, was cut from the brain and placed in 2 ml methanol to determine the TRH content.

Deiodinase assay

Livers were homogenized, and type I deiodinase activity was determined in the homogenate by analysis of the production of radioiodide from outer ring-labeled rT_3 (25). Type I deiodinase activity was measured in incubations of 1 μM $[^{125}I]rT_3$ for 20 min at 37 C with 50 μg/ml homogenate protein in 0.1 M phosphate buffer (pH 7.2), 2 mM EDTA, and 5 mM dithiothreitol by the method described by Fekkes *et al.* (26).

Hormone assays

Levels of TSH were measured by RIA using materials and protocols supplied by the NIDDK, with TSH RP-2 as standard. The RIA for TRH was performed with antiserum 4319 (final dilution, 1:10,000), as reported previously (27). Plasma T_3 and T_4 were estimated by specific RIAs in unextracted plasma, as described by Hermus *et al.* (18). The plasma FT_4 fraction was determined by means of the SPAC FT_4 assay kit (Byk-Sangtec Diagnostica, Dietzenbach, Germany) (28), and the plasma FT_4 concentration was calculated as the product of the total T_4 level and the FT_4 fraction. Plasma corticosterone was measured by RIA, as described by Sweep *et al.* (24). Intra- and interassay coefficients of variation for the assays varied between 3–17%.

Pro-TRH mRNA determination

Pro-TRH mRNA was measured by a ribonuclease (RNase) protection assay, using a labeled antisense complementary RNA (cRNA) probe. Total hypothalamic RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction (29). From each sample, 10 μg hypothalamic RNA were used in a RNase protection assay, as described previously by Sambrook *et al.* (30) with a few modifications. Hybridization was carried out overnight at 55 C; for the RNase digestion, 2 U/ml RNase-T1 and 0.2 μg/ml RNase-A (both from Boehringer, Mannheim, Germany) were used. The 1322-basepair (bp) *Eco*RI/*Pst*I rat pro-TRH complementary DNA (cDNA) insert in a pSP65 vector (31) was kindly provided by Dr. S. L. Lee (New England Medical Center Hospitals, Boston, MA). The cRNA probe was synthesized using fragment 981–1322 of rat pro-TRH cDNA as a template. This 351-bp *Rsa*I fragment was isolated after agarose gel electrophoresis. Variations in procedure were accounted for by normalizing to the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) expression in each sample, using a cRNA probe transcribed from a 410-bp *Pst*I/*Sau*A1 fragment of the cDNA inserted in pBlueScript KS(-) (Stratagene, La Jolla, CA). Under the digestion conditions used, the GAPDH signal consisted of 2 bands of about 310 and 320 nucleotides (Fig. 1). Autoradiographs were scanned densitometrically with a LKB 2222–020 UltraScan XL Laser Densitometer (Pharmacia LKB Biotechnology 1987, Bromma, Sweden). The peak areas, corresponding to the bands, were integrated by the computer. Results were calculated as the ratio between the integrated optical densities of pro-TRH and GAPDH mRNA, and expressed as a percentage of the mean of the respective control values.

Pituitary TSHβ mRNA measurement

Total pituitary RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction (29), and 20 μg RNA were subjected to denaturing agarose gel electrophoresis and blotted onto Hybond N$^+$ filter (Amersham International PLC, Aylesbury, United Kingdom). TSHβ cDNA (420 bp), inserted in the *Pst*I site of a pBR322 vector, was kindly provided by Dr. W. W. Chin (Brigham and Women's Hospital, Boston, MA) (32). After electroporation in DH5 cells, DNA was isolated and digested with *Pst*I. The DNA fragment was isolated after agarose gel electrophoresis. Northern blotting and random primed labeling of the TSHβ cDNA with [^{32}P]deoxy-ATP were performed according to the method of Sambrook *et al.* (30). Variations in loading were accounted for by normalizing to the β-actin mRNA content in each lane, which was measured by hybridization with a ^{32}P-labeled rat actin cDNA probe (Fig. 1). Autoradiographs were quantified densitometrically with a model 620 video densitometer using 2D Analyst II software (Bio-Rad, Richmond, CA). Results were calculated as the ratios between the integrated optical densities of TSHβ mRNA and β-actin mRNA, and expressed as a percentage of the mean of the respective control values.

Statistical analysis

Results are presented as the mean ± SEM. A nonparametric test (Wilcoxon matched pairs, signed ranks test) and analysis of variance for a repeated measures design were used to analyze the data. Provided that significant overall effects were obtained by analysis of variance, further comparisons between groups were made using Duncan's multiple range test. Differences were considered significant at $P < 0.05$.

Results

Infusion of IL-1 (4 μg/day) induced signs of physical discomfort in the animals, including piloerection and decreased physical activity, as observed on the first day after implantation of the pumps. This visually observable uneasiness gradually diminished and disappeared on day 2. Infusion of IL-6 at a dose of 15 μg/day did not induce signs of discomfort. Treatment of rats with saline did not perceptibly distress the animals.

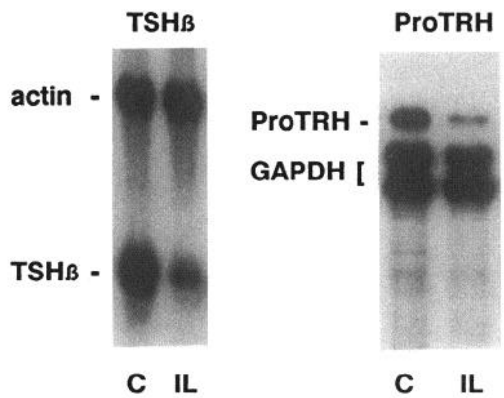

FIG. 1. Effect of continuous infusion of saline (C) or 4 μg IL-1/day (IL) for 1 week on pituitary TSHβ mRNA (*left panel*) and hypothalamic pro-TRH mRNA (*right panel*). Northern blot hybridization analysis was used to estimate TSHβ mRNA, whereas pro-TRH mRNA was determined with a RNase protection assay. Variation in loading was accounted for by normalizing to the β-actin mRNA and GAPDH mRNA contents, respectively.

Effects of IL-1 and IL-6 on rectal temperature and body weight

Saline-treated rats maintained a virtually constant mean daily rectal temperature throughout the experimental period. On the first day of infusion, IL-1 induced a significant increase in rectal temperature, which returned to normal levels between days 2–4 (Fig. 2), whereas IL-6-treated rats had no significant increase in rectal temperature compared to saline-treated rats.

There was a small decrease in body weight on the first day of saline infusion (Fig. 2). A similar weight loss was found in animals treated with IL-6 (15 μg/day), whereas rats infused with IL-1 (4 μg/day) showed a more distinct weight loss. The body weights of IL-1-treated rats reached minimal levels on the second day of infusion. Thereafter, the rate of body weight gain was slightly higher in IL-1-treated rats than in saline-treated control rats.

Effects of IL-1 and IL-6 on food and fluid intake

The effects of chronic administration of IL-1 and IL-6 on food and fluid consumption were monitored for 9 days, and results are shown in Fig. 3. There was a transient slight reduction in food consumption in saline-treated rats after implantation of the osmotic pumps. Compared to saline-treated animals, rats treated with IL-6 (15 μg/day) showed no significant change in food consumption, whereas the infusion of IL-1 (4 μg/day) caused a significant decrease in food intake compared to that in saline-treated rats during the first 5 days after starting the infusion. Chronic infusion of physiological saline, IL-1, or IL-6 into rats caused a significant decrease in total daily fluid intake on the first day of the infusion. During the following day, the fluid intake had returned to preinfusion values in all groups.

Effects of IL-1 on plasma T_4, FT_4, T_3, TSH, and corticosterone levels

Figures 4 and 5 show the effects of continuous infusion for 1 week with 4 μg IL-1/day or saline on plasma T_4, FT_4, T_3, and TSH. Infusion of 4 μg IL-1/day induced a highly significant decrease in plasma T_4, which reached minimum levels on day 2 and remained significantly suppressed throughout the experimental period. IL-1 induced a marked transient increase in the plasma FT_4 fraction (not shown), and the decline in plasma FT_4 in IL-1 rats was less pronounced and of shorter duration than that in total T_4. By the end of the infusion period, when plasma T_4 levels were still decreased, plasma FT_4 had returned to control levels. Parallel with the decrease in T_4 concentrations, plasma T_3 was significantly lower in IL-1-infused animals than in saline-treated rats. The nadir was reached on day 2 of the infusion, and plasma T_3 remained significantly lower in IL-1-treated ani-

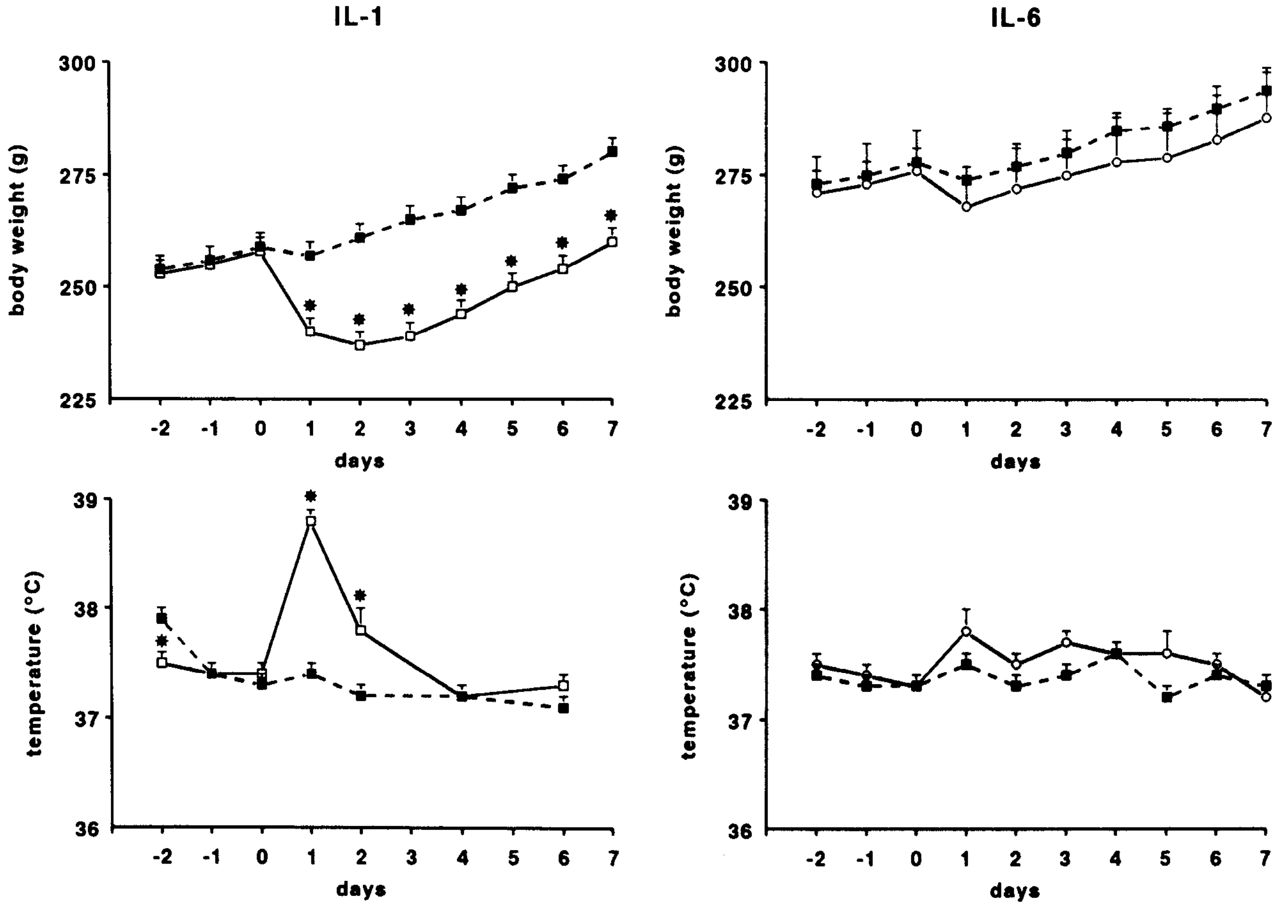

FIG. 2. Effects of continuous infusion of 4 μg IL-1/day (□), 15 μg IL-6/day (○), or saline (■) for 1 week on body weight and rectal temperature. Data are presented as the mean ± SEM of 7–17 rats. *, $P < 0.05$ compared to saline-infused rats.

mals than in saline-treated animals until the end of the experiment. Chronic administration of IL-1 induced a dramatic decline in plasma TSH. The nadir was reached on the first day of the infusion, after which plasma TSH levels in IL-1-treated rats started to increase slowly. Short term infusion with IL-1 had effects on thyroid function similar to those of long term infusion (Table 1). Levels of plasma corticosterone increased dramatically after 1 day of IL-1 infusion and were significantly elevated compared to levels in control rats for at least 4 days (Fig. 6).

Effects of IL-1 on pro-TRH mRNA, TSHβ mRNA, and type I deiodinase

In Table 2, the effects of treatment with IL-1 on hypothalamic pro-TRH mRNA, pituitary TSHβ mRNA, and liver type I deiodinase are given. During the first 2 days of infusion, the levels of hypothalamic pro-TRH mRNA in IL-1-treated rats were not significantly different from those in saline-treated rats. In addition, the TRH content in the median eminence did not change after 1 day of IL-1 infusion (Table 2). However, on day 7 of infusion, the level of hypothalamic pro-TRH mRNA was 73% lower in IL-1 rats than in controls. In the pituitary gland, the levels of TSHβ mRNA showed a significant decline after 2 days of IL-1 infusion. On days 2 and 7 of infusion, pituitary TSHβ mRNA levels were reduced to 38% of the levels in control rats. Liver type I deiodinase activity showed a significant decline due to IL-1 infusion on days 1, 2, and 7.

Effects of IL-6 on plasma T_4, FT_4, T_3, TSH, and corticosterone

Figures 4 and 5 show the effects of continuous infusion of rats for 1 week with IL-6 (15 μg/day) or saline on plasma T_4, FT_4, T_3, and TSH. Plasma T_4 was significantly lower in IL-6-infused animals than in control rats on days 2 and 3 of infusion. IL-6 produced a marked transient increase in the plasma FT_4 fraction (not shown), but plasma FT_4 in IL-6 rats did not change during the experiment. A significant decrease in plasma T_3 was found in IL-6-treated rats compared to their starting levels, but no significant effects were observed compared to saline-infused control values. Infusion of IL-6 induced a significant decline in plasma TSH. The nadir was reached on day 2 of the infusion, but plasma TSH recovered quickly, and within 4 days, the levels were again in the range found in control animals. Compared to the effects of IL-1 infusion on thyroid and pituitary function, the effects of IL-6 administration were less pronounced. This was also seen in the effects of these ILs on plasma corticosterone, because IL-6 administration did not affect the levels of plasma corticosterone, whereas IL-1 did (Fig. 6).

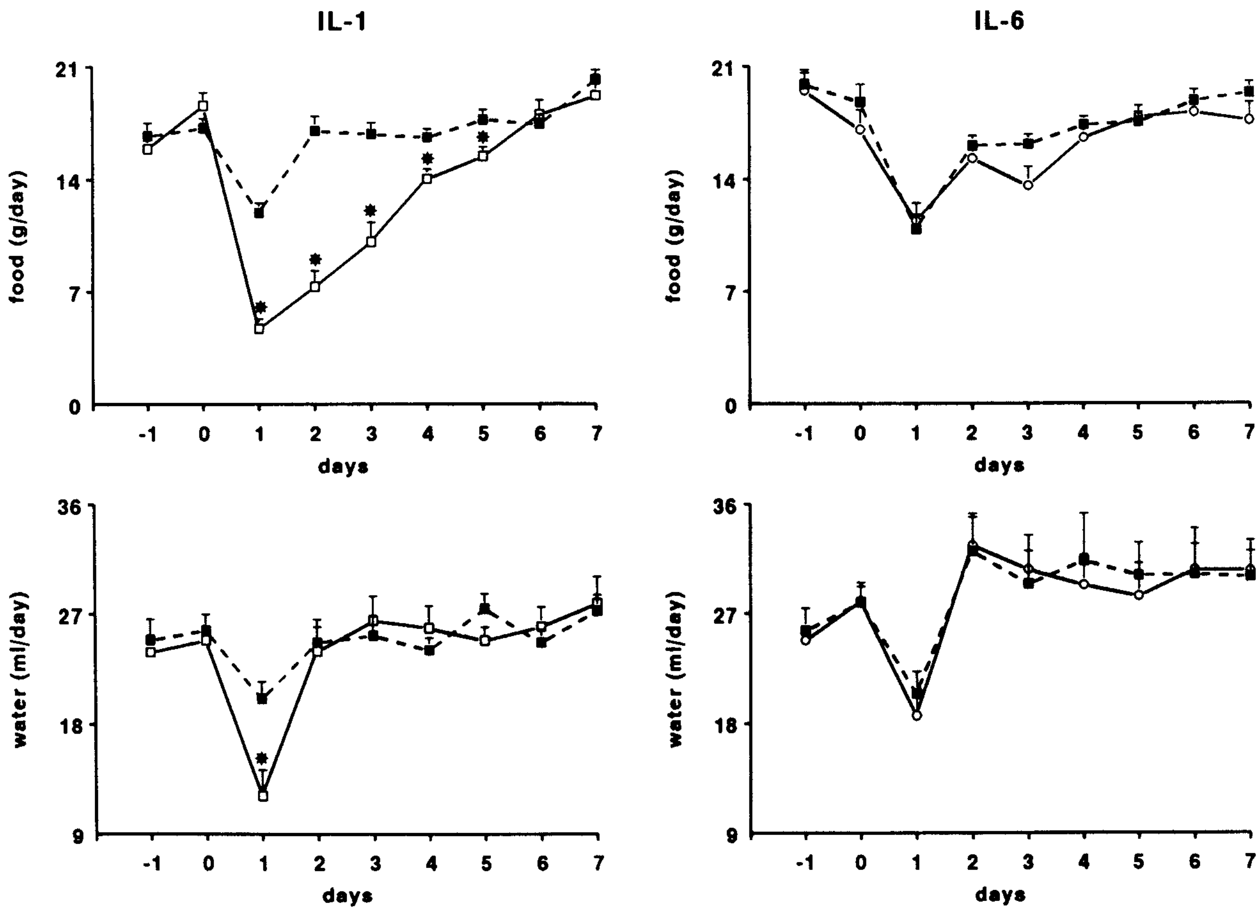

FIG. 3. Effects of continuous infusion of 4 μg IL-1/day (□), 15 μg IL-6/day (○), or saline (■) for 1 week on food and water intake. Data are presented as the mean ± SEM of 7–17 rats. *, $P < 0.05$ compared to saline-infused rats.

Effects of IL-6 on pro-TRH mRNA and TSH mRNA

In Table 3, the effects of continuous treatment with IL-6 on hypothalamic pro-TRH mRNA and pituitary TSHβ mRNA are shown. After 7 days of IL-6 administration, no effects were seen on the levels of hypothalamic pro-TRH mRNA. In the pituitary gland, the levels of TSHβ mRNA showed an insignificant decline after 7 days of infusion of IL-6.

Discussion

The suppressive effects of short term and continuous *in vivo* IL-1 administration on pituitary-thyroid function in rats have been reported in two previous studies (4, 18), in which it was shown that the reduction of food intake cannot explain the changes in thyroid hormone and TSH levels during IL-1 treatment (18). As the mechanisms of the effects of cytokine on thyroid function are not fully understood, we studied in particular the centrally mediated effects of IL-1α in more detail. Furthermore, as a number of IL-1 effects may be mediated by IL-6, we compared the effects of IL-1 and IL-6 infusions on plasma T_4, FT_4, T_3, TSH, and corticosterone; hypophysial TSHβ mRNA; median eminence content of TRH; and hypothalamic pro-TRH mRNA.

Infusion of both IL-1 and IL-6 produced a marked transient decrease in plasma T_4, which was more pronounced with IL-1 than with IL-6. Plasma FT_4 was also decreased by IL-1, but not by IL-6. These cytokines produced similar increases in the plasma FT_4 fraction (not shown), suggesting that IL-1 and IL-6 infusions both decreased plasma T_4 binding. Previous findings have shown that the decrease in plasma T_4 binding during IL-1 administration is due at least in part to a decrease in the plasma level of transthyretin, which is the principal plasma T_4-binding protein in rats (18). A decrease in transthyretin production is one of the hallmarks of the acute phase response of the liver to inflammation, which is largely mediated by IL-6 (33). As IL-1 is known to stimulate IL-6 production (33), it is likely that the effect of IL-1 on plasma T_4 binding is mediated by IL-6. However, besides the fall in plasma transthyretin a decrease in plasma albumin (33) and an increase in plasma FFA (4) may contribute to the lowered plasma T_4 binding during IL-1 and IL-6 administration.

The decrease in plasma FT_4 during IL-1 administration may be the result of a decrease in thyroidal T_4 production and/or an increase in plasma T_4 clearance. Dubuis *et al.* (4) demonstrated that plasma T_4 clearance is not affected by IL-1 administration despite a large increase in the plasma FT_4 fraction, suggesting that the metabolism of T_4 in the tissues

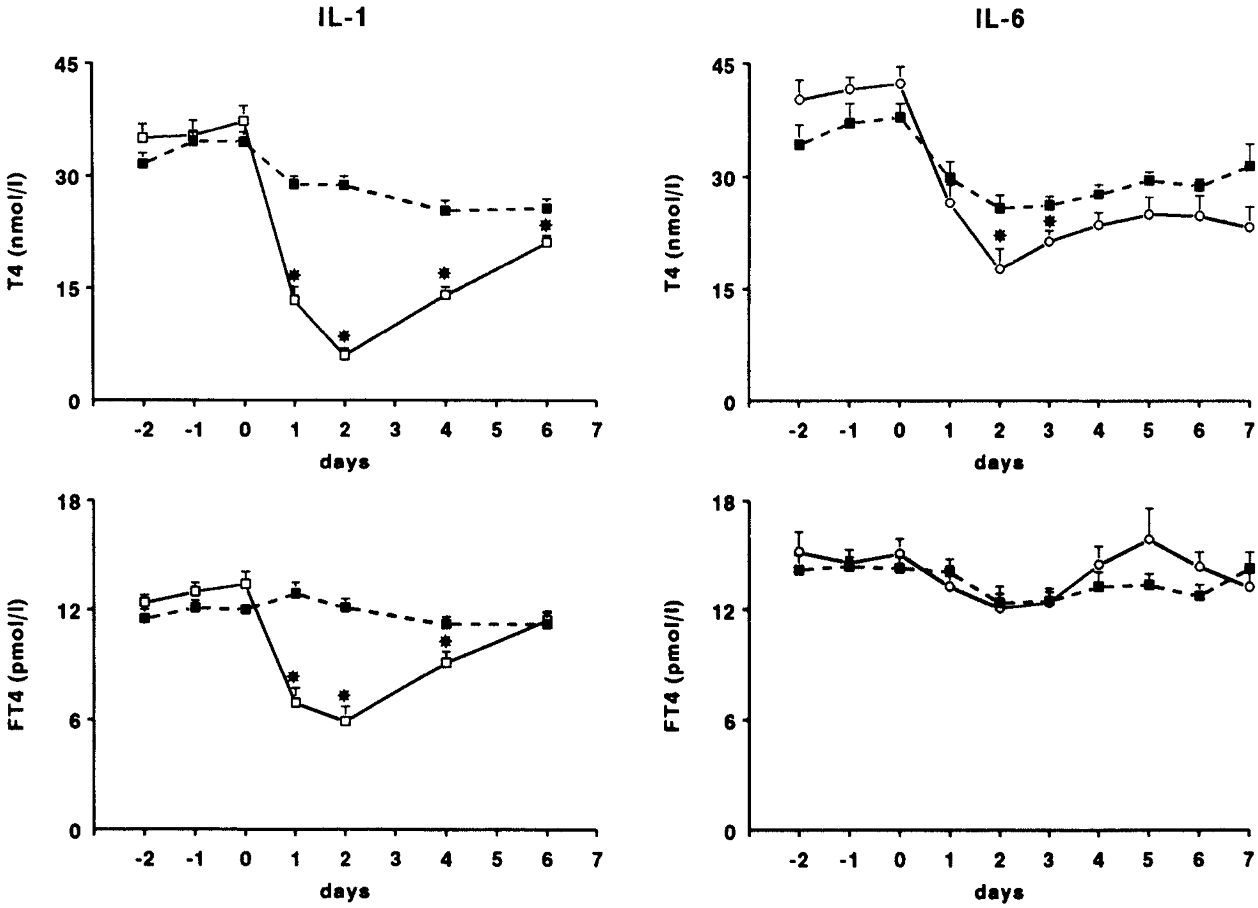

FIG. 4. Effects of continuous infusion of 4 μg IL-1/day (□), 15 μg IL-6/day (O), or saline (■) for 1 week on plasma TSH and T_3 levels. Blood samples were taken from an indwelling jugular venous cannula between 10–12 h. Data are presented as the mean ± SEM of 7–17 rats. *, $P < 0.05$ compared to saline-infused rats.

is decreased. This could be due to a decrease in tissue availability of plasma FT_4 or a decrease in the activity of T_4 metabolic pathways. Evidence has been presented that the fractional transfer rate constant for T_4 transport from plasma to liver is decreased in humans during severe illness and fasting (34, 35). Although changes in hepatic type I deiodinase activity have not been detected previously after both short and long term administration of IL-1 to rats (4, 18), significantly decreased deiodinase activities were found in the present study after 1, 2, and 7 days of IL-1 infusion. The reason for the differences in the effects of IL-1 on liver type I deiodinase between the previous (18) and the present studies could be due to the higher dose of IL-1 infused in the present study (4 *vs.* 2 μg/day). It should be stressed, however, that the decreases we observed were relatively small (~25%). It is not known to what extent these decreases were caused directly by an effect of IL-1 or IL-6 on the liver or indirectly through the IL-1-induced reduced food intake or hypothyroid state, which are both associated with a decrease in hepatic deiodinase activity (36). Surprisingly, infusion of mice with IL-1 for 3 days has been found to increase hepatic type I deiodinase activity, in contrast to the decrease found in animals with a similar reduction in food intake (7).

The reduced plasma T_4 and FT_4 levels induced by IL-1 in combination with a presumably normal plasma T_4 clearance rate, as found by others (4), suggest that IL-1 inhibits thyroidal T_4 secretion. The decrease in plasma T_3 during IL-1 administration may be due to 1) diminished T_3 secretion, 2) reduced peripheral T_3 production through a decrease in type I deiodinase activity and/or T_4 substrate availability, and/or 3) decreased plasma T_3 binding. An increased plasma FT_3 fraction was observed by Dubuis *et al.* (4) after IL-1 administration, although the effect was smaller than the increase in the plasma FT_4 fraction. IL-1 can inhibit thyroidal T_4 and T_3 secretion by a well documented direct effect on the thyrocyte, whereas IL-6 has little or no direct effect on thyroid activity (8, 37–40). However, the effects of IL-1 on thyroid function also appear to be mediated at least in part by the decrease in serum TSH.

In agreement with previous reports, IL-1 infusion resulted in a dramatic and acute decrease in serum TSH (4, 18), which was more rapid in onset and longer in duration than the decrease induced by IL-6. The latter may explain in part why, in contrast with IL-1, the decrease in serum TSH in IL-6-treated rats is not associated with a decrease in serum FT_4. Although the effects of cytokine administration on the clearance of plasma TSH have not been determined, the decreased serum TSH level probably reflects an acute decrease in

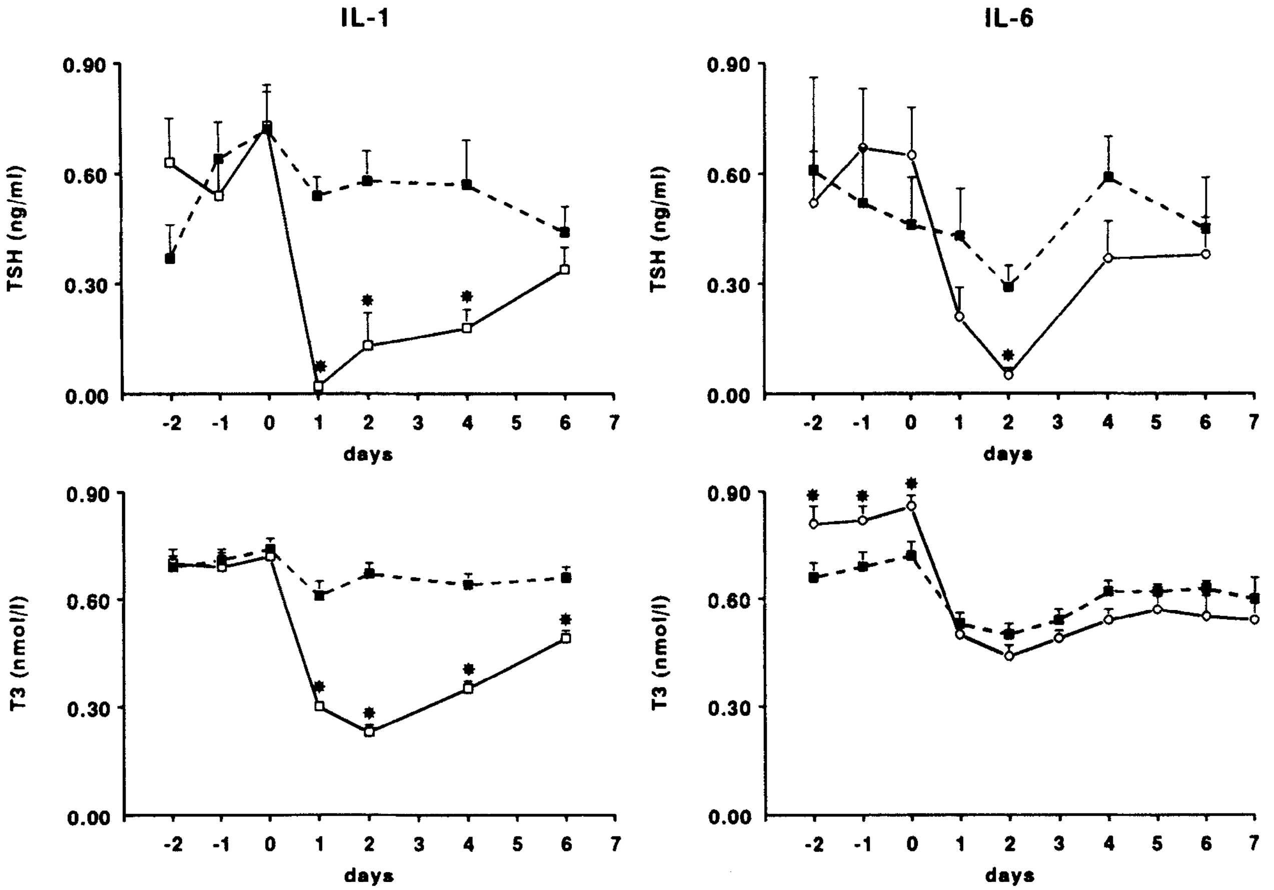

FIG. 5. Effects of continuous infusion of 4 μg IL-1/day (□), 15 μg IL-6/day (○), or saline (■) for 1 week on plasma T_4 and FT_4 levels. Blood samples were taken daily from an indwelling jugular venous cannula between 10–12 h. Data are presented as the mean ± SEM of 7–17 rats. *, $P < 0.05$ compared to saline-infused rats.

TABLE 1. The effects of continuous infusion of IL-1 (4 μg/day) or saline for 1 or 2 days were measured on plasma TSH, T_3, and T_4 levels in male rats

Parameter	Treatment	Day 1	Day 2
TSH (ng/ml)	Saline	0.38 ± 0.08	0.81 ± 0.09
	IL-1	0.11 ± 0.02[a]	0.06 ± 0.03[a]
T_3 (nmol/liter)	Saline	0.70 ± 0.06	0.61 ± 0.03
	IL-1	0.25 ± 0.02[a]	0.20 ± 0.02[a]
T_4 (nmol/liter)	Saline	40.7 ± 3.5	33.7 ± 1.4
	IL-1	12.3 ± 1.3[a]	4.5 ± 0.8[a]

Data are presented as the mean ± SEM of six or seven rats. After 1 or 2 days of infusion, trunk blood was collected after decapitation.

[a] $P < 0.05$ compared to saline-infused rats.

hypophyseal TSH secretion. This may be due to the direct effects of IL-1 and IL-6 on the thyrotroph or to alterations in hypothalamic or peripheral factors involved with TSH regulation. Concerning the latter, plasma FT_4 may be transiently increased acutely after commencement of cytokine administration, resulting in long-lived feedback inhibition of TSH secretion (4). It is remarkable, however, that hypophyseal TSHβ mRNA was not decreased after 1 day of IL-1 administration at the time serum TSH was at its nadir. Although this lack of an acute effect on hypophyseal TSHβ mRNA does not exclude a decrease in TSH synthesis, these results suggest that the IL-1-induced decrease in serum TSH after 1 day of IL-1 infusion is not secondary to a decreased TSH biosynthesis.

Inflammation in general and administration of cytokines such as IL-1 in particular have profound effects on multiple hypophyseal hormones, *e.g.* ACTH secretion is acutely increased (12–16, 24), whereas the secretions of TSH (4, 18), LH (17), and GH (41) are decreased. The effects of IL-1 on ACTH and LH secretion appear to be mediated largely by an increase in the hypothalamic production and secretion of CRF (42–44) and a decrease in the production and secretion of GnRH (45, 46), respectively. Evidence has also been presented that inhibition of GH secretion by IL-1 is due to an increased supply of hypothalamic somatostatin (47, 48). A suprahypophysial action of IL-1 on TSH secretion is supported by observations that intracerebroventricular administration of minute amounts of IL-1 produces a significant decline in plasma TSH in rats (49). The observation that not only basal serum TSH levels, but also their response to TRH stimulation are decreased during IL-1 infusion (18) suggests that a possible suprahypophysial effect of IL-1 on TSH secretion may be mediated by increased hypothalamic release of somatostatin, rather than decreased release of TRH. This is in agreement with the present findings that serum TSH

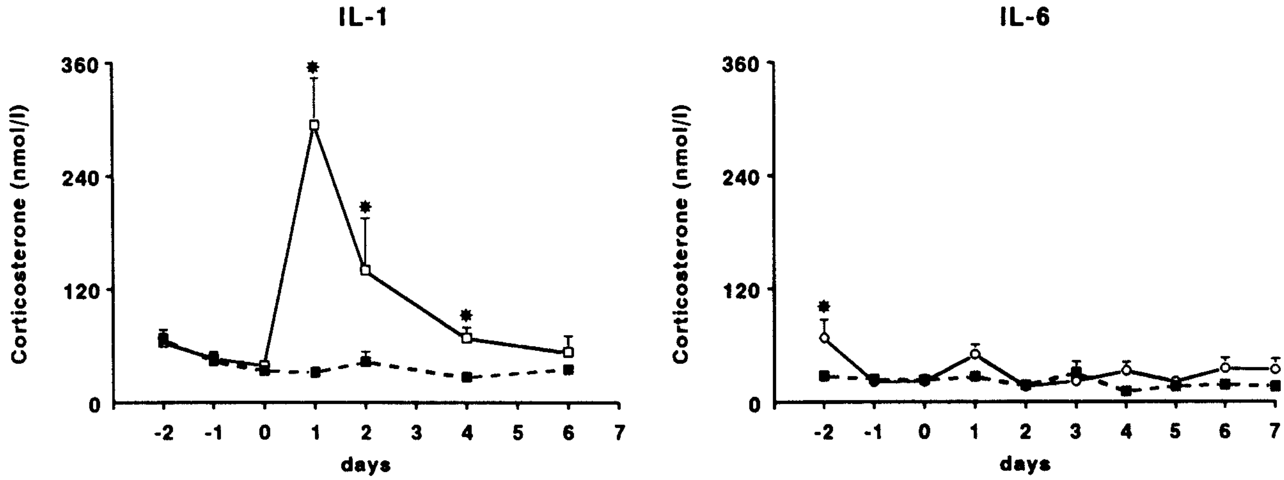

FIG. 6. Effects of continuous infusion of 4 μg IL-1/day (□), 15 μg IL-6/day (○), or saline (■) for 1 week on plasma corticosterone levels. Blood samples were taken daily from an indwelling jugular venous cannula between 10–12 h. Data are presented as the mean ± SEM of 7–17 rats. *, $P < 0.05$ compared to saline-infused rats.

TABLE 2. Effects of IL-1 (4 μg/day) infusion for 1, 2, or 7 days on the levels of hypothalamic pro-TRH mRNA, median eminence (ME) content of TRH, hypophysial TSHβ mRNA, and hepatic type I deiodinase in male rats

Parameter	Treatment	Day 1	Day 2	Day 7
Pro-TRH mRNA	Saline	100 ± 33	100 ± 25	100 ± 27
	IL-1	135 ± 17	125 ± 56	27 ± 7[a]
TRH in ME (ng)	Saline	1.3 ± 0.25	ND	ND
	IL-1	1.4 ± 0.28	ND	ND
TSHβ mRNA	Saline	100 ± 33	100 ± 14	100 ± 10
	IL-1	76 ± 30	38 ± 8[a]	38 ± 2[a]
Deiodinase (pmol/min·mg)	Saline	306 ± 25	208 ± 20	195 ± 13
	IL-1	243 ± 9[a]	159 ± 8[a]	123 ± 13[a]

Results are presented as the mean ± SEM ratios of the optical densities of pro-TRH mRNA over GAPDH mRNA or of TSHβ mRNA over β-actin mRNA, and expressed as a percentage of the mean of the respective control values. Groups contained five to nine rats. ND, Not determined.

[a] $P < 0.05$ compared to saline-infused rats.

TABLE 3. Effects of IL-6 (15 μg/day) infusion for 7 days on the levels of hypothalamic pro-TRH mRNA and hypophysial TSHβ mRNA in male rats

Parameter	Treatment	Day 7
Pro-TRH mRNA	Saline	100 ± 9
	IL-6	103 ± 22
TSHβ mRNA	Saline	100 ± 31
	IL-6	64 ± 7

Results are presented as the mean ± SEM ratios of the optical densities of pro-TRH mRNA over GAPDH mRNA or of TSHβ mRNA over β-actin mRNA, and expressed as a percentage of the mean of the respective control values. Groups contained six to eight rats.

and hypophyseal TSHβ mRNA are decreased before an effect of IL-1 is observed on hypothalamic pro-TRH mRNA. However, the lack of short term effects of IL-1 infusion on hypothalamic pro-TRH mRNA levels and median eminence TRH content does not exclude the possibility that IL-1 acutely inhibits TRH release into hypophyseal portal blood.

Direct effects of cytokines on anterior pituitary cells in culture have been reported, although this includes, paradoxically, stimulation of the secretion of TSH, LH, and GH (14). In this respect it is worthwhile to mention that both IL-1 and IL-6 are produced in the anterior pituitary and may, thus, act as paracrine factors in the regulation of hypophysial hormones (50, 51). In our study, IL-6 did not appear to act on the hypothalamus, as it failed to induce fever, nor did it stimulate the hypothalamic-hypophyseal-adrenal axis. It seems likely, therefore, that the effect of IL-6 on TSH secretion does not involve an action at the hypothalamic level, but, rather, a direct effect on the thyrotroph. As IL-1 induces the production of IL-6 (33), the effect of IL-1 infusion on TSH secretion may be mediated in part by this action of IL-6 on the pituitary.

As pro-TRH gene expression is only suppressed after 7 days of IL-1 infusion, it is likely that this effect is mediated by factors other than IL-1 itself. As discussed above, hypothalamic CRF gene expression is acutely stimulated by IL-1. As CRF neurons lie adjacent to TRH neurons in the paraventricular nucleus (PVN) (52, 53), the effects of IL-1 on TRH neurons may be mediated by local factors produced by CRF neurons. Kakucska *et al.* (54) showed by *in situ* hybridization a reduction of pro-TRH mRNA in the PVN 24 h after a constant intracerebroventricular infusion of IL-1, at the same time when pro-CRF mRNA in the PVN was increased. This inverse relationship between the levels of pro-TRH mRNA and CRF mRNA in PVN neurons has also been observed during hypothyroidism (55). Furthermore, high concentrations of glucocorticoids due to activation of the pituitary-adrenal axis may influence hypothalamic TRH production and secretion. In our study we demonstrated an increase in plasma corticosterone during at least 4 days of IL-1 infusion, whereas IL-6 infusion had no effect. A suppressive effect of plasma corticosterone on TRH gene expression would explain the different effects of IL-1 and IL-6 on pro-TRH mRNA. This hypothesis is supported by 1) the reduction in pro-TRH mRNA in the PVN after chronic high dose glucocorticoid treatment (56), 2) the presence of a consensus glucocorticoid response element in the TRH gene promoter (57), and 3) the coexistence of glucocorticoid receptors in TRH neurons in the PVN (58).

In conclusion, our findings suggest that in addition to the direct inhibition of thyroid hormone production by IL-1, the multiple effects of this cytokine on the hypothalamus-pituitary-thyroid axis include 1) a decrease in plasma T_4 binding; 2) an acute decrease in TSH secretion, followed by a decrease in TSH synthesis; and 3) only after prolonged IL-1 administration, a decrease in hypothalamic pro-TRH gene expression. The transient decrease in plasma T_4 binding and the acute decrease in TSH secretion are also observed during IL-6 infusion. The acute decrease in TSH secretion occurs before (IL-1) or even without (IL-6) a decrease in hypothalamic pro-TRH mRNA and, therefore, does not appear to be the result of decreased hypothalamic TRH synthesis, although a decrease in hypothalamic TRH release is not excluded. The decreased TSH secretion may also involve an increased supply of hypothalamic somatostatin as well as an effect via IL-6 directly on the thyrotroph. The decrease in pro-TRH gene expression by prolonged infusion of IL-1 may be mediated by the high plasma corticosterone levels.

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