Chronic infusion of TNF-α reduces plasma T4 binding without affecting pituitary-thyroid activity in rats

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Sweep, C. G. J., Mike J. M. van der Meer, H. Alec Ross, Roger Vranckx, Theo J. Visser, and Ad R. M. M. Hermus. Chronic infusion of TNF-α reduces plasma T4 binding without affecting pituitary thyroid activity in rats. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1099–E1105, 1992.—In the present study the effects of continuous administration of tumor necrosis factor-α (TNF-α), in a dose not affecting body temperature and food intake, on pituitary-thyroid function of rats were investigated. Male rats, bearing a venous catheter to allow repeated blood sampling, were intraperitoneally equipped with osmotic minipumps that continuously delivered recombinant human TNF-α (8.0 μg/day ip) or saline for 7 days. Infusion of TNF-α resulted in a significant decrease of plasma total thyroxine (T4) levels during days 2–5 of infusion as compared with the levels in saline infused rats. This suppression of plasma T4 concentrations was caused by a decreased binding of T4 in plasma, as indicated by an increased percentage of free T4. TNF-α infusion did not significantly affect free T3 levels in plasma nor basal and thyrotropin-releasing hormone-stimulated TSH levels. The decreased binding of T4 was, at least partially, caused by a reduction of T4-binding prealbumin (TBPA) levels in plasma, which were significantly reduced during the first 3 days of TNF-α infusion. Plasma levels of free fatty acids were not affected by TNF-α. TNF-α treatment did not influence the plasma 3,5,3′-triiodothyronine (T3)-to-T4 ratio nor hepatic 5′-deiodinase activity. Plasma reverse T3 levels remained undetectable both in control and TNF-α-treated rats. Taken together, our findings indicate that chronic infusion of rats with TNF-α in a subpyrogenic and subanorectic dose induces a transient decrease of plasma T4 binding without affecting pituitary-thyroid activity and peripheral thyroid hormone metabolism. The decrease in T4 binding is likely caused by a decrease in plasma TBPA levels. Our data support the concept that TNF-α may play a role in mediating changes in thyroid economy during systemic illness.

DURING ACUTE AND CHRONIC systemic illnesses, profound changes in thyroid function occur, both in humans (14, 31) and in animals (28). In humans, alterations during this “nonthyroidal illness syndrome” (NTI) include a decrease in the plasma 3,5,3′-triiodothyronine (T3) level while the reverse T3 (rT3) level is reciprocally increased. Low T3 and high rT3 levels in plasma are mainly due to a reduction of binding of T4 to carrier proteins (13, 19). Thyroid-stimulating hormone (TSH) concentrations in plasma of patients with systemic illness usually remain within the normal range but may be decreased (11, 25, 32).

Although mechanisms underlying NTI are probably multifactorial, it has been suggested that increased production of tumor necrosis factor-α (TNF-α), one of the polypeptides produced by macrophages and mononuclear cells in inflammatory sites, plays an important role in thyroid dysfunction in systemic illness (21, 22, 29). TNF-α administration in vivo considerably changes thyroid hormone levels in humans and laboratory animals (21, 22, 29). In humans, a single bolus injection of TNF-α (50 μg/m2) induced significant decreases in T3 and TSH levels and a significant increase in rT3 levels (29). Administration of TNF-α in mice in doses of 20 and 100 μg/day for 3 days decreased T4, free T3 (FT3), T3, and rT3 concentrations in a dose-dependent manner (21). A single injection of 200 μg TNF-α/kg body wt significantly reduced serum TSH, T4, FT4, and T3 levels in rats (22).

In the above-mentioned studies, TNF-α was administered in rather high doses as single or repeated bolus injections. During stimulation of the immune system, however, production of TNF-α is probably persistently enhanced. In the present study, we therefore determined the effects of continuous administration of a low dose of TNF-α on plasma levels of thyroid hormones and of TSH in the rat. TNF-α was continuously infused for 1 wk via osmotic minipumps implanted in the peritoneal cavity. In contrast to earlier studies, we used TNF-α in a dose that did not induce illness, a decrease in food intake, or an increase in body temperature. We found that infusion of rats with 8.0 μg TNF-α/day significantly reduces plasma T4 binding but not pituitary-thyroid activity. Furthermore, our data suggest that TNF-α infusion in the dose used did not cause significant alterations in peripheral thyroid hormone metabolism.

METHODS

Materials

Human TNF-α produced by DNA technology in Escherichia coli was obtained from Genentech (San Francisco, CA) through the courtesy of Dr. G. Adolf, Ernst-Boehringer-Institut for Arzneimittel Forschung (Vienna, Austria). The preparation has a specific activity of 6 × 107 U/mg protein (bioassay; murine L-M cells). According to the specifications of the supplier, the endotoxin contamination was negligible (<1.2 ng/mg protein as detected in the limulus amoebocyte lysate assay). The preparation

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chemicals used were of analytical grade. Experimental Procedures

Male albino Wistar rats (Cpb:WU, 10–12 wk of age, 200–220 g) were obtained from the local breeding facility. The animals were individually housed in Plexiglas cages on a 12:12-h light-dark cycle (lights on at 7:00 A.M., lights off at 7:00 P.M.). During the experiments the rats were fed ad libitum with commercial rat Chow (RMH-TM, Hope Farms, Woerden, The Netherlands), and they had free access to drinking water.

Animals

Male albino Wistar rats (Cpb:WU, 10–12 wk of age, 200–220 g) were obtained from the local breeding facility. The animals were individually housed in Flexiglas cages on a 12:12-h light-dark cycle (lights on at 7:00 A.M., lights off at 7:00 P.M.). During the experiments the rats were fed ad libitum with commercial rat Chow (RMH-TM, Hope Farms, Woerden, The Netherlands), and they had free access to drinking water.

Experimental Procedures

Starting 1 wk before cannulation, the rats were handled daily by the experimenter between 9:30 and 11:30 A.M. to minimize the stress response to manipulation. Blood was collected from freely moving rats by means of a chronic cannula. The animals were anesthetized with Hypnorm (0.5 ml/kg body wt im; 10 mg/ml fluanisone and 0.315 mg/ml phentanyll citrate, Janssen Pharmaceutica, Tilburg, The Netherlands)-midazolam hydrochloride (5.0 mg/kg body wt ip; Hoffmann-La Roche, Mijdrecht, The Netherlands)-atropine (0.05 mg/rat sc; Pharmachemie, Haarlem, The Netherlands). Under sterile conditions, a Silastic cannula (0.5 mm ID; 0.94 mm OD; with a knotting) was inserted into the right external jugular vein and passed down to the atrium. The distal end of the cannula was tunneled subcutaneously and exteriorized through a stab wound in the skin of the head, where it was connected to a hooked stainless steel tube. This assembly was anchored to the skull with stainless steel screws and acrylic cement. During cannulation, rats were continuously exposed to a gas flow of O2-N2. To keep the catheter patent, 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, FRG).

After 7–9 days, all animals were equipped with osmotic minipumps (Alzet, Palo Alto, CA), which are capable of delivering a continuous flow of 1 nl/h for 7 days (model 2001). The pumps were filled with either sterile pyrogen-free 0.9% sodium chloride or TNF-α in saline. After loading, they were equilibrated in saline at 37°C and implanted intraperitoneally in ether-anesthetized animals (3:00–5:00 P.M.). One group of rats (n = 6) was continuously infused at a rate of 8.0 μg TNF-α·24 h⁻¹·rat⁻¹, whereas a control group of rats (n = 6) received osmotic pumps filled with 0.9% pyrogen-free saline. The indwelling cannula and the osmotic pumps were tolerated well by the animals, with no signs of discomfort or infection.

Blood was collected from freely moving rats daily for 9 days, starting 1 day before implantation of the pumps (day 1 up to and including day 7). Because of the circadian variation in hormone release, blood was withdrawn from the animals every day at about the same time (between 10:00 and 12:00 A.M.). Blood samples (2.0 ml) were collected in prechilled tubes containing EDTA (60 μl of a 10% EDTA solution in saline). Blood samples were gently shaken and spun for 10 min at 1,500 g (4°C). Plasma was separated, and red blood cells were resuspended in sterile physiological saline (1.5 ml) and returned to each rat. Plasma samples were aliquoted and stored at -20°C until assayed.

Protocol

In a pilot experiment (data not shown), we investigated the effects of continuous infusion of rats with 4.0 and 8.0 μg TNF-α/day on plasma levels of total T₄, on food intake, daily body weight change, and on rectal temperature. TNF-α (4 μg/day) induced a slight decrease of plasma T₄ levels, whereas 8.0 μg/day was more effective. Both treatments had no significant effect on food intake, daily body weight change, and rectal temperature. Thereafter the effects of chronic treatment of rats with 8.0 μg TNF-α/day on plasma levels of total T₄, FT₄, total T₃, rT₃, TSH, free fatty acids (FFA), and T₄-binding prealbumin (TBPA) were investigated. In addition, on days 2 and 6 of infusion, thyrotropin-releasing hormone (TRH) tests were performed. A bolus injection of synthetic TRH (500 ng/rat; Roche Diagnostica, Hoffmann-L.a Roche) was given through the jugular cannula to the animals between 10:00 and 12:30 A.M. Blood samples (0.7 ml) for measurements of plasma TSH concentrations were taken immediately before and 20 min after TRH injection. On day 7 after starting the infusion, rats were killed by decapitation, the livers were perfused through the portal vein with physiological saline, cut into pieces, and frozen in liquid nitrogen. The liver microsomal type 15-deiodinase activity was measured in incubations of 1 μM 3',5'-[125I]T₃ for 20 min at 37°C with 50 μg/ml homogenate protein in 0.1 M phosphate (pH 7.2) and 2 and 5 mM EDTA by the method of Fekkes et al. (10). Body weight was measured daily at 8:00 A.M., and food and water intake was estimated at approximately the same time by weighing the residual food and water for individual cages. Body temperature was measured serially two times per day between 8:30 and 9:00 A.M. and between 1:00 and 2:30 P.M. 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). The mean daily temperature for each rat was determined by averaging the morning and afternoon rectal temperatures. The changes in body temperature were determined by subtracting the preinfusion measurements (mean temperatures of days 1 and 0) from the values on the respective infusion days.

Hormone Assays

Plasma TSH. Highly purified rat TSH (rTSH) for iodination (AFP-7308C), rabbit anti-rTSH antiserum (C21381), and rTSH reference preparation (AFP-5153B) for radioimmunoassay of rTSH were kind gifts of the National Institute of Diabetes and Digestive and Kidney Diseases, obtained through the courtesy of Dr. S. Raiti of the National Hormone and Pituitary Program, University of Maryland, School of Medicine. Tracer was prepared by lactoperoxidase radioiodination, and [125I]-rTSH was subsequently separated from free iodine on a C25 column and further purified on a G100 column. Suppressed TSH plasma was obtained by injecting rats with 20 μg T₃ daily for 3 days and 1 μg T₃ 1 h before death. Plasma samples giving the least displacement of tracer binding to the antiserum were pooled and employed in the standard curve. rTSH was estimated in duplicate in 100-μl aliquots of rat plasma. Standard concentrations corresponded to 0.25–100 ng/ml. Antiserum was added to a final dilution of 1:10,000, and incubation took place at 4°C for 24 h, after which tracer was added followed by another 24 h of incubation. Antibody-bound radioactivity was separated by addition of sheep anti-rabbit antiserum in a 7.5% polyethylene glycol solution. All necessary comparisons between test and control animals were made within one assay run. Optimal within-assay precision of 4.8% (coefficient of variation of duplicate means) was obtained at 2.2 ng/ml. The limit of detection was 0.14 ng/ml.

Plasma total T₄ and FT₄. Radioimmunoassay of rat plasma total T₄ was performed using rabbit anti-T₄ antiserum purchased from Henning (Berlin, FRG) to a final dilution of 1:32,000 and T₄ tracer from Amersham (Houten, The Netherlands). Hormone-free rat plasma was prepared by repeatedly mixing rat plasma with charcoal (15 g/100 ml) and centrifuging. The procedure for removal of T₄ from rat plasma was the same as that routinely applied in our laboratory for stripping human serum, in which T₄ is more tightly bound. The latter procedure
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Fig. 1. Effect of continuous infusion of rats with tumor necrosis factor-α (TNF-α; 8.0 pg/day; closed circles) or saline (open circles) for 1 wk on plasma concentrations of thyroxine (T4), free T4 (FT4), percentage FT4 (%FT4), 3,5,3'-triiodothyronine (T3), and the ratio T3/T4. Blood samples were taken daily between 10:00 and 12:00 A.M. Osmotic minipumps containing TNF-α or saline were implanted ip on day 0 (arrow). Data are presented as means ± SE of 5 rats.

*P < 0.05, **P < 0.005, and ***P < 0.0005 statistically significant differences between TNF-α- and saline-treated rats.

has been tested for its efficacy of removing T4 by means of tracer addition and for the absence of charcoal fines by microscopic examination. The assay was performed on 5-μl samples. Standards to which 5-μl hormone-free plasma was added ranged from 12.5 to 200 nmol/l. After 2 h of incubation at room temperature the antibody-bound radioactivity was separated by means of sheep anti rabbit antiserum in polyethylene glycol. The average limit of detection was 11 nmol/l. Within-assay and between-assay coefficients of variation of duplicate means were 3.0 and 2.4%, respectively, at an average level of 50 nmol/l.

FT4 in rat plasma was estimated indirectly, i.e., by means of multiplication of assay results from separate measurements of total T4 and the percentage of plasma FT4 (%FT4). The latter was estimated by means of the SPAC FT4 assay kit (Byk-Sangtec Diagnostica, Dietzenbach, FRG). The principle of this assay is as follows: plasma and T4 tracer are equilibrated with low-affinity high-capacity monoclonal anti-T4 antiserum, which is coupled to the tube wall. The ratio FT4/plasma protein-bound T4 tracer equals by definition the fraction FT4 or %FT4. By virtue of the characteristics of the antibody, the ratio free/antibody-bound tracer is independent of the total T4 and PT4 concentration and thus is a constant, its magnitude being dependent on the properties of the antibody exclusively. Therefore the ratio antibody-bound tracer/plasma protein-bound tracer is directly proportional to the FT4 fraction. The proportionality factor cancels out by using a standard sample with a known FT4 fraction. Within-assay and between-assay coefficients of variation of duplicate means were 4.2 and 5.8%, respectively, at an average level of 15.4 pmol/l.

Plasma T3 and rT3. Radioimmunossay of rat plasma total T3 was performed using Amerlex M total T3 kits from Amersham (Houten, The Netherlands) with modified standard curves. These contained 50 μl hormone-free rat plasma (see above) and 0.125-4 nmol/l T3. The average limit of detection was 0.063 nmol/l. Within-assay and between-assay coefficients of variation of duplicate means were 6.8 and 7.2%, respectively, at an average level of 0.65 nmol/l.

Plasma rT3 levels were determined according to the method described by Visser et al. (30). The limit of detection of this assay was 0.05 nmol/l.

Plasma Concentrations of TBPA and FFA

Plasma TBPA levels were measured according to Bleiberg et al. (2). Plasma concentrations of FFA were measured by an enzymatic colorimetric test (NEFA C) using the acyl-coenzyme A (CoA) synthetase acyl-CoA oxidase 3-methyl-N-ethyl-N-(β-hydroxyethyl)aniline method (Wako Chemicals, Neuss, FRG).

Statistical Analysis

From each group, one rat had to be excluded because of blood clotting in the cannula. All data are presented as means ± SE of five rats in each group. Comparisons between the control and the TNF-α group were made by analysis of variance (ANOVA) with repeated measurements (days 1–7). Only when ANOVA revealed a statistically significant difference was comparison of the individual groups at specific time points further evaluated by Student's t test for unpaired observations.

RESULTS

Effects of TNF-α on Plasma Thyroid Hormone Levels

Figure 1 shows the effects of continuous infusion of rats for 1 wk with 8.0 μg TNF-α/day or with saline on plasma
levels of T₄, FT₄, %FT₄, T₃, and the T₃-to-T₄ ratio. Pre-infusion values (days 1 and 0) were not different between the treatment groups. Infusion of rats with TNF-α induced a highly significant decrease in plasma T₄ levels (ANOVA, P < 0.005). T₄ levels during TNF-α infusion were lowest on day 2 (17.7 ± 1.0 vs. 31.9 ± 3.3 nmol/l, TNF-α vs. saline, P < 0.005), remained significantly depressed up to and including day 5 (22.6 ± 1.2 vs. 33.6 ± 1.1 nmol/l, P < 0.0005), and were still lower at day 7 (19.8 ± 1.3 vs. 25.9 ± 2.4 nmol/l, P = 0.052). There was only a slight decline in FT₄ levels in plasma of TNF-α-treated rats, which did not reach statistical significance. The %FT₄ in plasma of rats treated with TNF-α was significantly increased as compared with values in control animals (ANOVA, P < 0.005). The increase was maximal on day 2 (0.0586 ± 0.0025 vs. 0.0372 ± 0.0015%, TNF-α vs. saline, P < 0.0005), and %FT₄ levels remained significantly elevated up to and including day 4 (0.0441 ± 0.0012 vs. 0.0337 ± 0.0015%, P < 0.005). Thereafter %FT₄ in plasma of TNF-α-treated rats was within the control range. Parallel with the decrease in T₄ concentrations, plasma T₃ levels were significantly lower (ANOVA, P < 0.005) in TNF-α-infused animals as compared with saline-treated rats. The nadir was reached on day 2 of infusion (0.51 ± 0.02 vs. 0.86 ± 0.02 nmol/l, TNF-α vs. saline, P < 0.0005), and plasma T₃ levels remained lower until the end of the experiment. The T₃-to-T₄ ratio in rats treated with TNF-α did not significantly differ from that in saline-treated rats throughout the treatment period.

Effects of TNF-α on Basal and TRH-Stimulated Plasma TSH Levels

Figure 2, top, shows the plasma concentrations of TSH during TNF-α and saline infusion. Chronic administration of 8.0 μg TNF-α/day induced a slight decrease in plasma TSH levels, which, however, did not reach statistical significance (ANOVA, P = 0.40).

The response of the pituitary gland to TRH was measured on days 2 and 6 of infusion with TNF-α and saline. In both groups, the concentration of TSH in plasma increased ~10-fold after an intravenous bolus injection of 500 ng TRH on day 2 as well as on day 6 (Fig. 2, bottom). There was no difference in response to TRH between TNF-α- and saline-treated rats.

Effects of TNF-α on Liver 5'-Deiodinase

and on Plasma Levels of rT₃

5'-Deiodinase activity in liver homogenates of TNF-α-treated rats was not significantly different from that in saline-treated rats (521 ± 29 vs. 542 ± 16 pmol·min⁻¹·mg protein⁻¹, TNF-α vs. saline). Circulating levels of rT₃ were lower than the limit of detection of the assay (P < 0.05 nmol/l) at all days of the study, both in TNF-α- and saline-treated rats.

Effects of TNF-α on Plasma Levels of TBPA and FFA

Figure 3 shows that chronic infusion with TNF-α induced a significant reduction in plasma TBPA levels as compared with values in saline-infused rats (ANOVA, P < 0.005). TBPA levels were already significantly lower on day 1 (337 ± 17 vs. 456 ± 28 μg/ml, TNF-α vs. saline, P < 0.05), reached a nadir on day 2 (223 ± 6 vs. 413 ± 31 μg/ml, P < 0.0005), and returned to control levels on day 4 (385 ± 49 vs. 465 ± 47 μg/ml, not significant). Due to sample shortage, plasma FFA levels could not be measured on days 2 and 6 of infusion. FFA concentrations in plasma samples of TNF-α-treated rats did not differ from those in control rats.

Effects on Food Intake, Body Weight, and Rectal Temperature

Before the start of the infusion the two groups consumed similar amounts of food and were equal in weight. There was a moderate decrease in food consumption and in body weight on the day of osmotic pump implantation in saline- and TNF-α-treated rats, as compared with pre-infusion values. Infusion of rats with TNF-α did not notably affect food intake and daily body weight change, as compared with saline infusion. Daily fluid intake was not influenced by treatment of rats with TNF-α, as compared with controls (data not shown).

Changes in rectal temperature were calculated for each rat by subtracting the mean preinfusion value of each rat from the temperatures on the respective infusion days. As shown in Fig. 4, bottom, treatment of rats with TNF-α did not significantly affect rectal temperature, as compared with values in saline-treated animals.
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DISCUSSION

In the present study, we have investigated the effects of continuous intraperitoneal infusion of TNF-α by osmotic minipumps on plasma thyroid hormone levels in rats. We demonstrated that infusion of TNF-α in a dose as low as 8.0 μg/day caused a profound decrease of plasma T₄ and T₃ concentrations. However, the FT₄ concentration in plasma of TNF-α-treated rats did not change significantly, as compared with values in saline-infused animals. No significant effect of TNF-α treatment was found on basal plasma TSH concentrations nor on TSH responsiveness to TRH. Together, our data show that infusion of TNF-α induced a reduction in T₄ binding in plasma, whereas the activity of the pituitary-thyroid axis was not significantly affected.

In the adult rat, plasma T₄ is predominantly bound to TBPA and to a lesser extent to albumin (7, 27). Binding to T₄-binding globulin has also been observed in some circumstances (7). In the present study, we showed that TNF-α infusion caused a transient decline of T₄ binding, as indicated by the increased percentage of FT₄. In parallel with the inhibition of T₄ binding, plasma levels of TBPA were decreased in rats treated with TNF-α. Plasma TBPA levels were already significantly diminished on day 1, reached their nadir on day 2, and returned to control levels on day 4. Therefore, it is likely that the diminished T₄ binding in TNF-α-treated rats is, at least partially, due to a decrease in the plasma concentration of TBPA.

The plasma level of TBPA decreases in response to the challenge of acute inflammation (8, 26). Studies in adult rats have suggested that the plasma protein response to inflammation is triggered by hormones and cytokines. Considerable interest has been focused on TNF-α in this respect. TNF-α injections in laboratory animals induce synthesis of acute phase proteins by the liver, whereas albumin levels are decreased in TNF-α-treated animals (9). Mice inoculated with hamster ovary cells transfected with the gene for human TNF-α manifested decreased serum albumin levels, albumin synthesis, and albumin...
mRNA levels (3). Most probably, TNF-α affects albumin gene expression by the liver directly, since TNF-α decreases albumin synthesis in primary rat hepatocyte cultures (15) and albumin mRNA levels and albumin synthesis in human hepatoma cell lines (23, 24). In parallel with the effect on albumin synthesis, it might be that TNF-α acts directly on liver cells to inhibit prealbumin synthesis. Alternatively, it might be that TNF-α induces an increase in the turnover of TBPA.

A moderate decrease in serum protein binding of T₄ is commonly found in patients with the nonthyroidal illness syndrome (1, 19). In these patients the diminished binding of T₄ appears to be partially caused by a fall in the concentrations of binding proteins (19). These changes, however, do not appear to account entirely for the altered T₄ binding (33), and the induction of a circulating factor that inhibits T₄ binding to plasma proteins has been postulated (5, 18). In humans, plasma FT₄ levels increased transiently after a bolus injection of TNF-α (29).

This suggests that TNF-α reduces plasma concentrations of T₄ binding proteins or induces a factor in plasma that may interfere with binding of T₄. FFA have been suggested to displace T₄ from its binding proteins in plasma, causing an increase in the FT₄ fraction (4, 12). Moreover, FFA exhibit thyroid hormone binding inhibitor activity in a ligand-binding assay in vitro (6). Interestingly, Van Der Poll et al. (29) recently showed that a bolus injection of TNF-α caused a significant increase in plasma levels of FFA. In the present study, we found that FFA levels in plasma of TNF-α-treated rats did not change, excluding the possibility that FFA substantially contributed to the reduction of T₄ binding.

Repeated administration of TNF-α in rodents induces a rapid development of tolerance to the toxic and anorectic effect of the cytokine (10, 17). Similarly, it may be that plasma TBPA concentrations and T₄ binding are only transiently decreased during TNF-α infusion because of induction of tolerance to TNF-α. Although cytokines are reported to remain bioactive in osmotic minipumps, (20) we cannot exclude, however, that the decrease of the effect of TNF-α on TBPA levels and T₄ binding is caused by a loss of bioactivity of TNF-α in the pumps during the course of the experiment.

TNF-α treatment has been reported to modulate peripheral thyroid hormone metabolism. Liver type I 5’-deiodinase activity in rats was reduced 8 h after a single bolus injection of 200 μg TNF-α/kg but had increased, as compared with control levels, after the third of successive daily injections of 50, 200, and 800 μg TNF-α/kg (22). Administration of 20 μg TNF-α/day for 3 days to mice resulted in a significantly higher T₃-to-T₄ ratio and in decreased rT₃ levels in plasma, whereas hepatic 5’-deiodinase activity in TNF-α-treated mice was significantly higher than in pair-fed control animals (21). In humans, TNF-α treatment induced a significant increase in rT₃ levels (29). In our study, rT₃ plasma levels remained below the limit of detection of the assay. We did not find any effect of infusion of 8.0 μg TNF-α/day on the plasma T₃-to-T₄ ratio and on hepatic 5’-deiodinase activity, suggesting that this dose of TNF-α did not affect peripheral thyroid hormone metabolism.

The present observations are consistent with previous reports in which a decline in plasma levels of total T₄ after administration of TNF-α to rodents has been described (21, 22). We did not observe a significant effect of TNF-α on plasma TSH and FT₄ levels. Pang et al. (22) demonstrated that a single injection of 200 μg TNF-α/kg significantly reduced serum TSH and fT₄ levels, as compared with the values in saline-injected rats. In experiments with repeated injections in a dose of 150 μg TNF-α·kg⁻¹·day⁻¹ for 5 days, however, serum TSH levels reverted to normal. In mice, administration of TNF-α in a dose of 20 μg/day for 3 days decreased serum FT₄ concentrations, as compared with pair-fed control animals (21). In humans, intravenous bolus injections of TNF-α (50 μg/m²) induced a decrease in plasma TSH levels, whereas T₃ and FT₄ levels were not significantly affected (29). Taken together, treatment of laboratory animals and humans with high doses of TNF-α has been reported to decrease the activity of the pituitary-thyroid axis. In the present study, we showed that continuous infusion with a dose of TNF-α as low as 8.0 μg/day for 1 wk did not affect the activity of the pituitary-thyroid axis in rats.

Acute administration of TNF-α to human volunteers (29) or laboratory animals (21, 22) reproduces many of the thyroid function changes, as commonly seen in systemic illness. These observations have led to the suggestion that alterations in thyroid economy during systemic illness may be related to elevated serum TNF-α concentrations (21, 22). In these studies, pharmacological doses of TNF-α were used, however, and the animals and humans were visibly ill after TNF-α administration. It is therefore not clear whether the hormonal changes observed after treatment with these doses of TNF-α reflect direct effects of TNF-α per se or are secondary to other effects induced by the cytokine. In the present study, we showed that continuous infusion with 8.0 μg TNF-α/day had no effect on the activity of the pituitary-thyroid axis and on peripheral thyroid hormone metabolism, whereas a transient reduction in plasma binding of T₄ was induced. Because this dose of TNF-α did not affect food intake, body weight, and rectal temperature, the observed alterations in T₄ binding during TNF-α infusion probably reflect a direct effect of TNF-α per se.

We thank G. Greuters from the Central Animal Laboratory for expert biotechnical assistance. Profs. P. W. C. Kloppenborg, Th.J. Benraad, and A. G. H. Smals are acknowledged for critically reading the manuscript.

This work was supported by the Royal Netherlands Academy of Arts and Sciences.

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Received 2 April 1992; accepted in final form 9 July 1992.

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