Interleukin 6 effects on the pituitary–thyroid axis in the rat

Luigi Bartalena, Lucia Grasso, Sandra Brogioni and Enio Martino

Istituto di Endocrinologia, University of Pisa, Tirrenia-Pisa, Italy


It has been postulated recently that cytokines, and in particular interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α), may have a role in the pathogenesis of the changes of serum thyroid hormone concentrations that are encountered in patients with non-thyroidal illness (NTI). Many of the IL-1 and TNF-α effects are believed to be mediated by the induction of IL-6 synthesis, which might, therefore, represent an important mediator of thyroid hormone changes in NTI. To address this problem, male Wistar rats were injected subcutaneously with 2.5 μg of recombinant human IL-6 (rhIL-6, in 500 μl of saline solution), with 2.5 μg of rhIL-6 preincubated with 100 μl of anti-IL-6 neutralizing antibody or with saline solution alone (control group). Administration of rhIL-6 resulted in a significant decrease of thyroxine (T₄) from 82 ± 4 nmol/l (mean±sem) to a nadir of 33 ± 3 nmol/l (p < 0.0001) after 48 h, and of triiodothyronine (T₃) from 1.6 ± 0.1 to 0.8 ± 0.1 nmol/l after 48 h (p < 0.0001). A slight decrease in serum T₄ and T₃ concentrations also was observed in the control group, but the lowest values (T₄, 66 ± 3 nmol/l; T₃, 1.2 ± 0.1 nmol/l) were significantly higher (p < 0.0001) than in IL-6-treated rats. The IL-6-induced changes could be prevented by preincubation of rhIL-6 with its neutralizing antibody. Slight but not significant changes occurred in serum reverse T₃ (rT₃) concentration, so that the T₄/rT₃ ratio remained substantially unchanged after rhIL-6 injection, whereas the T₄/T₃ ratio decreased significantly from 53.6 to 39.9 (p < 0.02) in IL-6-treated rats. The effects of IL-6 on thyrotropin (TSH) were investigated after rendering the rats hypothyroid by methimazole administration for 3 weeks. Serum TSH decreased from 19.0 ± 6.8 to 13.3 ± 3.8 μg/l after 48 h (p < 0.01) in IL-6-treated rats, while it increased from 17.2 ± 2.8 to 25.8 ± 4.0 μg/l (p < 0.01) in the control group. These results show that a single injection of rhIL-6 causes a decrease in serum T₄, T₃ and TSH concentrations in the rat, without affecting serum rT₃ levels. This is compatible with a predominantly central effect of the cytokine. The apparent lack of inhibition of 5'-deiodinating activity, a key feature of NTI, suggests that IL-6, if involved, is only one of the factors responsible for the changes of thyroid hormone secretion and metabolism observed in NTI.

Luigi Bartalena, Istituto di Endocrinologia, University of Pisa, Viale del Tirreno 64, 56018 Tirrenia-Pisa, Italy

Changes in serum concentrations of thyroid hormones, which occur in non-thyroidal illnesses (NTI) and define the so-called “Euthyroid Sick Syndrome” (ESS), are well characterized and include a decrease in serum triiodothyronine (T₃), an increase in serum reverse T₃ (rT₃) and, in most severe forms, a decrease in serum thyroxine (T₄) concentrations (1). The pathogenesis of these abnormalities is far less understood, but circumstantial evidence has been provided that cytokines, and especially interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α), might be factors involved in their regulation (1). Recently, the elevated serum IL-6 concentration found in NTI has been correlated with the abnormalities of thyroid function tests found in ESS, suggesting also that this cytokine might participate in the pathogenesis of these changes (2, 3).

The role of IL-6 in generating abnormalities of thyroid function in vivo in the animal is controversial, because preliminary reports have provided evidence that thyroid function tests are affected by IL-6 in rats (4) but not in mice (5). Thus, the present study was undertaken to evaluate further the effects of IL-6 administration on the pituitary–thyroid axis in the rat.

Materials and methods

Animals

Adult (6-week-old) male Wistar rats weighing 150–200 g were housed, four per cage, in plexiglass cages in an artificially lighted and temperature-controlled room (lights on at 07.00 h, lights off at 19.00 h), and fed rat chow and tap water ad libitum for 2 weeks prior to the experiments.

Test materials

Recombinant human IL-6 (rhIL-6) expressed in Escherichia coli and goat anti-rhIL-6 neutralizing antiserum were obtained from R&D Systems (Berkeley, USA).
anti-rhIL-6 antibody had no cross-reactivity with IL-1 or TNF-α.

**In vivo experimental procedure**

On the day of the experiment, rats were weighed and anesthetized by ether. In a preliminary experiment, rats were subdivided into four groups of six rats, receiving subcutaneously either saline solution alone, rhIL-6 in three different doses (1, 2.5, or 5 µg dissolved in 500 µl of saline solution) or normal goat serum (100 µl added with 400 µl of saline solution). Blood was drawn from the tail, under slight anesthesia, prior to injection and 4 and 24 h after. In three subsequent experiments, rats were divided into three groups of eight rats: the first group was administered a single subcutaneous injection of rhIL-6 (2.5 µg in 500 µl of saline solution); the second group received rhIL-6 preincubated for 1 h at room temperature with 100 µl of undiluted goat anti-IL-6 neutralizing antiserum; the third group was constituted by control rats injected with saline (500 µl). Blood was drawn, as described above, prior to injection and 4, 24, 48 and 72 h after. Serum was stored at −20°C until assayed.

In three subsequent experiments, hypothyroidism was induced in two additional groups of eight rats by the addition of methimazole to the drinking water (1.5 g/l) 3 weeks prior to the experiment. Rats received either rhIL-6 (2.5 µg dissolved in 500 µl of saline) or saline alone. Blood was drawn prior to the experiment and 24 and 48 h after.

**Assays**

Radioimmunoassay (RIA) kits were used for the determination of serum total T4 and T3 (Byk Gulden, Milan, Italy) and rT3 (Ares Serono, Milan, Italy); the latter assay had a sensitivity of 0.019 nmol/l. Serum TSH concentration was determined by RIA using the material kindly provided by the National Hormone and Pituitary Program, National Institutes of Health, Bethesda, USA, as described previously (6).

**Statistical analysis**

Values were expressed as means ± SEM. Undetectable rT3 values were given, for statistical purposes, an arbitrary value equal to one half of the least detectable value. Variations within groups were analyzed by one-way ANOVA for repeated measures and by Student–Newman–Keuls test for multiple comparisons. Differences between different groups were analyzed by Student’s t-test for unpaired data.

**Results**

In preliminary experiments, the subcutaneous injection of 1 µg of rhIL-6 was associated with a slight, but not significant, reduction in serum T4 and T3 concentrations, while the administration of 2.5 and 5 µg caused a significant decrease in hormone levels. As rats given 5 µg showed a pronounced reduction of the body weight, a dose of 2.5 µg of rhIL-6 was selected for further experiments. The injection of normal goat serum did not produce variations of serum T4 and T3 levels different from those observed in control rats injected with saline.

In a subsequent experiment, rats were treated with 2.5 µg of rhIL-6, with 2.5 µg of rhIL-6 preincubated with 100 µl of anti-IL-6 neutralizing antibody or with saline solution alone. Throughout the experiment, the food intake did not differ in the three groups: accordingly, a similar decrease in body weight was observed in control rats (from 211 ± 10 to 195 ± 8 g), IL-6-treated rats (from 205 ± 8 to 196 ± 10 g) and rats treated with IL-6 preincubated with the neutralizing antibody (from 197 ± 6 to 190 ± 7 g), with no differences among groups. As shown in Fig. 1, a single subcutaneous injection of 2.5 µg of rhIL-6 caused a significant decrease in serum T4 from 82 ± 4 to 62 ± 3 nmol/l after 4 h (p < 0.001), 41 ± 3 nmol/l after 24 h (p < 0.0001) and 33 ± 3 nmol/l after 48 h (p < 0.0001); at 72 h values were slightly, although not significantly, higher than after 48 h (43 ± 3 nmol/l). A slight, but significant, decrease was observed also in the control group (saline alone) from 80 ± 3 to 66 ± 3 nmol/l (p < 0.01) after 72 h. Baseline serum T4 values did not differ in IL-6-treated and in control rats, whereas values after 4, 24, 48 and 72 h were

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**Fig. 1.** Changes in serum T4 concentrations (mean ± SEM of three experiments) in rats given subcutaneously recombinant human interleukin 6 (2.5 µg of rhIL-6 in 500 µl of saline solution) (▲), rhIL-6 preincubated with 100 µl of anti-IL-6 neutralizing antibody (○) or saline solution alone ( []). *p < 0.001 vs baseline; **p < 0.0001 vs baseline; ***p < 0.01 vs baseline.
significantly lower (p < 0.0001) in rats receiving rhIL-6. As illustrated in Fig. 1, the effect of rhIL-6 could be prevented by preincubating the cytokine with 100 µl of anti-IL-6 neutralizing antibody: in this group of rats, serum T3 decreased from 75 ± 5 to 64 ± 2 nmol/l after 48 h (p < 0.01), with no significant differences with respect to the control group.

As shown in Fig. 2, a similar decrease, after rhIL-6 administration, was observed for serum T3 concentration from 1.6 ± 0.1 to 0.8 ± 0.1 nmol/l (p < 0.0001) after 48 h. The control group also showed a slight, but not significant, decrease in serum T3 levels, from 1.5 ± 0.1 to 1.2 ± 0.05 nmol/l after 72 h (p = NS). As for T4, the IL-6 effect on T3 could be abolished by preincubating the cytokine with its neutralizing antibody (Fig. 2).

Slight, but not significant, variations in serum rT3 concentration were found in either group (from 0.048 to 0.044 nmol/l in control group, from 0.045 to 0.035 nmol/l in IL-6-treated rats, and from 0.044 to 0.042 nmol/l in the group treated with the neutralized cytokine). Two rats in each group had undetectable serum rT3 levels, which remained undetectable throughout the experiment. Accordingly, the T4/rT3 ratio showed only marginal and not significant changes throughout the experiment, with no differences between control and IL-6-treated rats (Table 1). At variance, the T4/T3 ratio decreased significantly from 53.6 to 39.9 (p < 0.02) in IL-6-treated rats but not in control rats or in rats treated with the neutralized cytokine (Table 1).

Euthyroid rats had low-normal or undetectable serum TSH values, which did not allow detection of small changes. Therefore, to investigate the effects of IL-6 on TSH secretion, rats were made hypothyroid by adding methimazole to the drinking water for 3 weeks prior to injection with either rhIL-6 or saline. Hypothyroidism was confirmed by the very low or undetectable serum T4 and T3 concentrations. In the control group, serum TSH increased from 17.2 ± 2.8 to 25.8 ± 4.0 µg/l after 48 h (p < 0.001), whereas in IL-6-treated rats the hormone concentration decreased from 19.0 ± 4.8 to 13.3 ± 3.8 µg/l (p < 0.01). Thus, in control rats a 50% increase in serum TSH concentration was observed during the 48-h experiment, while in the IL-6 group a 30% decrease occurred (Fig. 3). Baseline serum TSH levels did not differ, but values at 48 h were significantly lower in IL-6-treated rats (p < 0.0001).

**Discussion**

Interleukin 6 is a pleiotropic cytokine involved in B-cell terminal differentiation and activation of T cells (7), stimulation of acute-phase proteins (7) and regulation of hormone transport proteins (8, 9). It is synthesized by different cells, including thyroid cells (10-12). Increased serum IL-6 concentrations have been found in patients with thyroid destructive processes, such as subacute thyroiditis (13), some forms of amiodarone-induced thyrotoxicosis (14) and after percutaneous
ethanol injection into hot nodules (15). Under these circumstances, the increased IL-6 concentration in serum is the expression of thyroid cell damage.

Serum IL-6 levels are increased also in patients with NTI (16–21), and appear to correlate with changes of serum thyroid hormone concentration (2, 3). Furthermore, administration of IL-6 to patients with metastatic renal carcinoma has been associated with a reduction in serum T₃ and TSH concentrations and an increase in serum rT₃ levels (22). Thus, IL-6 might represent a possible mediator of ESS.

Our results in vivo in the rat showed that a single rhIL-6 injection is followed by a decrease in serum T₄, T₃ and TSH concentrations. These data are in keeping with preliminary results by Onoda et al. (4) in the rat, but in disagreement with negative effects of IL-6 in mice (5). It is possible that these discrepancies may be attributed to the different animal species. Our study demonstrated that IL-6 affects TSH secretion (at least in hypothyroid animals), but the mechanism responsible for this action is unclear. Onoda et al. (4) failed to show any substantial change in pituitary TSH content and TSH-β mRNA abundance, or in the hypothalamic TSH-releasing hormone content. It might, therefore, be that the inhibitory effect of IL-6 is indirect, carried out through other IL-6-stimulated mediators, such as ACTH and adrenal steroids (23). Interestingly, IL-6 has been reported to stimulate in vitro the release of other anterior pituitary hormones, including prolactin, growth hormone and luteinizing hormone (24). Whatever the mechanism(s), the inhibition of TSH secretion by IL-6 is in agreement with the notion that in NTI, despite the reduction in serum thyroid hormone concentration, serum TSH is often inappropriately low/normal (with a reduction or abolishment of its nocturnal peak), thus depicting a condition of central hypothyroidism (25–28).

The reduction in serum T₄ and T₃ concentrations might be the consequence of the decreased thyroid stimulation by TSH and/or of a direct effect of IL-6 on the thyroid. While the first possibility is likely in view of the cytokine effect on TSH, the second mechanism could not be demonstrated directly in our animal model. However, it is worth mentioning that IL-6 seems to have direct effects at the thyroid level, where it has been reported to block TSH-induced thyroid hormone release and thyroid peroxidase gene expression (29). It is, therefore, conceivable that the reduction in thyroid hormone release induced by IL-6 in vivo may be mediated partly by an IL-6-induced reduction in TSH release, but also may result from a direct action of the cytokine at the thyroid level. It should, however, be mentioned that IL-6 failed to influence thyroglobulin production by cultures of human thyrocytes (30).

Interestingly, IL-6 administration was associated neither with an increase in serum rT₃ concentrations nor with a significant decrease in the T₄/rT₃ ratio. This is in agreement with the report by Hermus et al. (31), who found serum rT₃ levels below the limit of detection of the assay throughout IL-1β infusion. At variance, the T₄/T₃ ratio decreased significantly after IL-6 injection. These data, taken together, suggest that type I deiodinase activity is not reduced by IL-6 administration in the rat. In this regard, it is worth noting that IL-1 and TNF-α have been reported to increase 5'-deiodinating activity in the mouse liver (32, 33).

Both central (i.e. a reduced TSH secretion) and peripheral (i.e. a reduced peripheral monodeiodination of T₄) mechanisms intervene in the pathogenesis of ESS (see Ref. 1 for a review). Interest in the role of cytokines in NTI has focused mostly on TNF-α and IL-1, and multiple effects of these cytokines have been described both at the hypothalamic–pituitary level and at the thyroid level (31–36), which might be mediated partially by induction of IL-6 synthesis and release (7). However, while our results showed that IL-6 does inhibit TSH (at least in hypothyroid animals) and thyroid hormone secretion, they apparently failed to demonstrate any impairment of 5'-deiodinating activity, which is a key feature of ESS. It is likely, therefore, that IL-6, if involved, is only one of the (largely unknown) factors responsible for the changes of thyroid hormone secretion and metabolism seen in NTI.

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