Influence of interleukin 1 and antihuman interleukin 1 receptor antibody on the growth and function of the thyroid gland in rats

G Żerek-Meleń, K Żylińska, J Fryczak, S Mucha and H Stepień

Department of Experimental Endocrinology and Hormone Diagnostics, Institute of Endocrinology, Medical University of Łódź, Łódź, Poland


Cytokines seem to influence the hypothalamic–pituitary–thyroid axis. We have studied the effect of different doses of interleukin 1α (IL-1α) and IL-1β (given twice daily ip) alone or together with antihuman IL-1 receptor antibody (aIL-1ra) on the proliferation of thyroid follicular cells and thyroid hormone levels in male Wistar rats. We have examined the influence of IL-1α and IL-1β at doses of 10.0, 1.0 and 0.1 μg/kg body wt of animal and aIL-1ra at a dose of 10.0 μg/kg body wt of animal. The incorporation of bromodeoxyuridine into thyroid follicular cell nuclei was used as an index of cell proliferation (labeling index: LI) and measured 24 h after the last of two injections of interleukin. Interleukin 1β, at all examined doses, increased thyroid follicular cell proliferation when compared to controls (p < 0.05), and a positive correlation between log of the dose of IL-1β used and LI (r = 0.62, p < 0.05) using Student’s t-test was found. The administration of aIL-1ra alone also enhanced the thyroid follicular cell proliferation, whereas aIL-1ra used together with IL-1β exerted a less pronounced effect than each of these substances used separately (p < 0.05). Interleukin 1α at the dose of 10.0 μg/kg body wt increased the proliferation of thyroid follicular cells (p < 0.05). Thyroid hormone levels did not change in any of the experiments. These results suggest a regulatory role of IL-1 upon the proliferation of thyroid cells.

Henryk Stepień, Institute of Endocrinology, Medical University of Łódź, Dr. Sterling Str. 3, 91-425 Łódź, Poland

Many diseases (infections, tissue damage) are associated with a variety of immunological and endocrine changes, with the alteration of serum thyroid hormone levels, called “euthyroid sick syndrome”, mediated at least partially by the effect of cytokines (1). Interleukin 1 (IL-1) is one of the representative cytokines possessing a wide range of biological activity, and it is produced by activated macrophages and other cell types (2, 3). The name IL-1 refers to two proteins, IL-1α and IL-1β, which are encoded by separate genes and show limited homologies at the amino acid level (4). However, they bind to the same cell-surface receptor and show similar biological activity (5). Besides the functional role of IL-1 in the immune system, IL-1 has the capacity to affect cell growth and differentiation in both normal and neoplastic cell types, including astrocytes, bone cells, fibroblasts, monocytic leukemia cells, renal mesangial cells and human thyroid carcinoma cell line NIM 1 (6–11). It has been shown that some cells possess IL-1 receptors among other human thyroid cells (12). Dubuis et al. (13) have found that subcutaneous injections of IL-1β decreased plasma thyroxine (T₄), triiodothyronine (T₃) and thyrotropin (TSH) levels in rats. Continuous infusion (for 1 week) of various doses of IL-1β to rats caused a significant decrease in plasma free T₃, free T₄ and TSH levels and impaired TSH responsiveness to TRH administration (14). Others have observed that plasma T₃ and T₄ responses to TSH seem to be weaker in IL-1-treated rats (15). Enomoto et al. (16) have found that the long-lasting inhibitory effect of IL-1 on the thyroid might be independent on pituitary TSH content. So far, the effect of IL-1 on the thyroid cell growth has not been established; some authors have shown the stimulatory influence of IL-1 on human thyroid cell growth, whereas others have indicated its inhibitory effect (12, 17).

The aim of the present paper was to determine the effect of different doses of recombinant human IL-1α and IL-1β and antihuman IL-1 receptor antibody (aIL-1ra), given separately or simultaneously, on thyroid growth and function.

Materials and methods

Male Wistar rats, weighing 100 ± 10 g were used in the study. The animals were kept under controlled light conditions (12 h light, 12 h darkness) with food and water ad libitum.

The animals were divided into ten groups (10 animals in each group) and treated as follows:

Group 1: control, 0.9% NaCl ip;
Group 2: IL-1α (Immunex Research Development
Corporation, Seattle, USA; specific activity $1 \times 10^6$ U/mg protein), 10.0 µg/kg body wt. ip; Group 3: IL-1α, 1.0 µg/kg body wt. ip; Group 4: IL-1α, 0.1 µg/kg body wt. ip; Group 5: IL-1β (Immunex Research Development Corp.; specific activity $1 \times 10^6$ U/mg protein), 10.0 µg/kg body wt. ip; Group 6: IL-1β, 1.0 µg/kg body wt. ip; Group 7: IL-1β, 0.1 µg/kg body wt. ip; Group 8: all-1ra (Hu IL-1R; M10; Immunex Research Development Corp.), 10.0 µg/kg body wt. ip; Group 9: IL-1α, 10.0 µg/kg body wt. + all-1ra, 10.0 µg/kg body wt. ip; Group 10: IL-1β, 10.0 µg/kg body wt. + all-1ra, 10.0 µg/kg body wt. ip.

Animals in each group received ip injections twice daily. The first injection was given at 20.00 h, the second one at 08.00 h on the following day. On the second injection the animals additionally received bromodeoxyuridine (Brdu; Sigma, St Louis, MO) ip at a dose of 50 mg/kg body wt. Ninety minutes later the animals were killed by decapitation and thyroid glands and blood samples were collected from each animal. Tissues were embedded in paraffin wax and the paraffin sections were immunostained using cell proliferation kit (Amersham International, Amersham, Bucks, UK) to detect Brdu incorporated into thyroid cell nuclei. The labeling index (LI), expressed as the number of Brdu-immunopositive nuclei per 1000 randomly scored thyroid follicular cells, was estimated in microscopic preparations (six to eight sections from each thyroid gland). All semiserial cut sections were estimated, one being chosen for counting. The incorporation of Brdu into cell nuclei is a simple repetitive morphological method used to measure cell proliferation. This method is more specific for our purposes than the stathmokinetic or [$^3$H]thymidine incorporation methods, as it excludes the measurement of fibroblast cell proliferation ($^3$H]thymidine uptake) and piknotic cell (stathmokinetic method). Thyroid hormone levels were measured by commercially available RIAs (Halex, Poland, in coproduction with Byk–Sangtec Diagnostica, Germany). The data were analyzed statistically and the significances of differences between medians of LIs were determined by Mann–Whitney’s test. The data of T₄ and T₃ plasma levels were analyzed statistically by means of ANOVA and Student’s t-test.

Results

Changes in LIs were measured in the presence of different doses of interleukins to find out the effect of IL-1α and IL-1β on the growth of thyroid follicular cells. Interleukin 1β at all examined doses (10.0, 1.0 and 0.1 µg/kg body wt) enhanced the LIs of thyroid follicular cells (TFC) as compared to controls. The administration of all-1ra also significantly increased the LI of TFC. However, all-1ra given together with IL-1β at a dose of 10.0 µg/kg body wt caused a smaller proliferogenic effect than either of these substances used separately (Fig. 1). A positive correlation between log of the dose of IL-1β and LIs was found (r = 0.62, p < 0.05) using Student’s t-test.

The administration of IL-1α at a dose of 10.0 µg/kg body wt increased the proliferation of TFC. Similarly to IL-1β, the use of all-1ra together with IL-1α at a dose of 10.0 µg/kg body wt exerted a less pronounced
It seems that IL-1β could exert the proliferogenic effect either directly through the IL-1 specific receptors or indirectly by various growth factors, such as IGF-I, prostaglandins and cAMP (20, 21). Recently, other mechanisms involved in the thyroid cell growth and differentiation, such as the inhibition of phospholipase A2 activity, calcium mobilization and the regulation of ß2-adrenergic receptors, have been postulated (22, 23).

In addition, thyroid follicular cells may also produce a substance with an IL-1-like bioactivity (12, 24). Moreover, in autoimmune thyroid diseases (e.g. Graves disease and Hashimoto’s thyroiditis), inflammatory macrophages and lymphocytes might be a source of IL-1 (25). Thus, the possible local paracrine and/or autocrine mechanism of IL-1 proliferogenic action cannot be excluded. In conclusion, we have shown that peripheral administration of IL-1β stimulates a dose-dependent proliferation of thyroid follicular cells in rats in vivo. However, further studies are needed in order to determine the precise mechanism of cytokine action on the growth of the thyroid gland.

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**References**

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13. Dubois JM, Dayer JM, Siegrist-Kaiser CA, Burger AG. Human recombinant interleukin-1β decreases plasma thyroid hormone

**Table 1. The influence of IL-1α, IL-1β and antihuman IL-1 receptor antibody (aIL-1ra) on the blood serum level of triiodothyronine (T₃) and thyroxine (T₄).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood serum level (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>T₃, 2.18 ± 0.16 T₄, 51.48 ± 5.02</td>
</tr>
<tr>
<td>IL-1α, 10μg/kg</td>
<td>T₃, 2.09 ± 0.15 T₄, 54.31 ± 3.73</td>
</tr>
<tr>
<td>IL-1β, 1.0μg/kg</td>
<td>T₃, 2.12 ± 0.07 T₄, 48.78 ± 1.67</td>
</tr>
<tr>
<td>IL-1α, 0.1μg/kg</td>
<td>T₃, 1.80 ± 0.11 T₄, 48.26 ± 3.35</td>
</tr>
<tr>
<td>IL-1β, 0.1μg/kg</td>
<td>T₃, 1.85 ± 0.05 T₄, 51.48 ± 2.83</td>
</tr>
<tr>
<td>IL-1α, 0.1μg/kg</td>
<td>T₃, 1.81 ± 0.14 T₄, 50.97 ± 1.93</td>
</tr>
<tr>
<td>IL-1β, 0.1μg/kg</td>
<td>T₃, 1.85 ± 0.08 T₄, 52.90 ± 2.83</td>
</tr>
<tr>
<td>aIL-1ra</td>
<td>T₃, 1.94 ± 0.13 T₄, 41.06 ± 3.47</td>
</tr>
</tbody>
</table>

*Values are means ± SEM.

Discussion

Measurements of in vivo BrdU incorporation into DNA have been developed recently and applied to the study of normal and transformed cells kinetics. Bromodeoxyuridine, an analogue of thymidine, is specifically taken up by cells in the DNA synthetic phase (S-phase) of the cell cycle. Therefore, cells in the S-phase can be identified by immunocytochemical staining with anti-BrdU monoclonal antibody after labeling with BrdU (18, 19). Our data clearly show that both IL-1α and IL-1β stimulated thyroid follicular cell proliferation in vivo. On the other hand, the two cytokines tested in the same experimental conditions did not change the thyroid hormone secretion. Our findings suggest an important regulatory role of IL-1 in the growth control of the thyroid gland in rats. Additionally, they confirm earlier postulated interactions between IL-1 and the thyroid, which may exist in various diseases. The indicated proliferogenic effect of IL-1 is concordant also with the data obtained by other authors (11, 12). On the contrary, Kimura et al. (17) have shown an inhibitory effect of IL-1 on thyroid growth. These inconsistent data are probably caused by different experimental conditions, including various animal models and the way of cytokine administration. Although IL-1 is known to be a proliferogenic factor for some tissues, the mechanism of its action is unclear.

Neither IL-1α and IL-1β nor aIL-1ra, used separately or together, changed T₄ or T₃ levels (Table 1).

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