Retinoic acid differentially modulates triiodothyronine and retinoic acid receptors in rat liver according to thyroid status

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Triiodothyronine (T₃) receptors (TRs) and retinoic acid (RA) receptors (RARs) exert their effects on growth, differentiation and cellular homeostasis by acting as transcription factors. The binding characteristics of these receptors have been studied in liver of hypothyroid and hyperthyroid rats, with or without treatment with T₃, RA or T₃ + RA together. The changes in binding induced by RA treatment depended on the hormonal status of the rat. In hypothyroid rats the T₃ binding capacity was unaltered by administration of T₃ or RA alone but increased by 48% after treatment with T₃ and RA together. In these rats administration of RA, T₃ or T₃ + RA increased the RAR binding capacity by 45, 79 and 112%, respectively. In hyperthyroid rats the administration of RA reduced the TR and RAR binding capacities by 22 and 37%, respectively. We found also that the affinity constants of TRs and RARs were reduced in hypothyroid rats after treatment with T₃ or T₃ + RA. It is suggested that this change of the properties of receptors is related to a ligand-dependent conformational change in these receptors.

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Retinoic acid (RA), an active metabolite of vitamin A (retinol), and other hormones involved in vertebrate growth, differentiation and cellular homeostasis exert their effects via nuclear receptors belonging to a superfamily of thyroid–steroid hormone receptors and by acting as transcription factors (1). The triiodothyronine (T₃) receptors (TRs) and the RA receptors (RARs) belong to the same subclass owing to their high degree of amino acid sequence homology and an identical P box in the first zinc finger. These receptors are represented by several isoforms encoded by corresponding genes. Studies performed mainly with transformed cell lines have shown that these receptors are subject to both homologous regulation (regulation of a receptor by its own ligand) and heterologous regulation (regulation by a ligand that is not its own ligand). Moreover, it was shown that the receptors form homodimers (TR–TR or RAR–RAR) or heterodimers (TR–RAR), and the dimer that binds the response elements of genes induces specific transcription products (2). Recent studies have suggested that TRs and RARs require interaction with RXRs (whose ligand is the 9-cis RA) for efficient binding and transactivation (3, 4). Thus, the multiplicity of potential interactions between receptors and ligands greatly increases the spectrum of cellular phenomena involved in signalling pathways and the variety of possible responses.

It has been shown previously that there is a thyroid hormone response element (THRE) in the gene of malic enzyme (MDH) (5) and that the cellular activity of MDH is controlled by T₃ (6). In addition, Duester et al. demonstrated that the expression of an alcohol dehydrogenase (ADH) gene (ADH-3) in human liver is induced by RA (7). These authors have suggested that RA activation of ADH-3 constitutes a positive feedback loop regulating RA synthesis. Thus, ADH activity could be considered as an indicator of RA action in cells.

In previous studies performed with intact animals we investigated the effect of vitamin A status on the properties of some nuclear receptors. Retinoids exert their action on the properties of TRs at either a transcriptional or a post-translational level. It was shown that an inadequate level of dietary retinol results in a decreased level of TR mRNA and subsequently in the decreased binding capacity of TRs (8, 9). Furthermore, RA administration induces an increased activity of protein kinase C (PKC) and subsequently an increased apparent affinity of the receptors for their ligand (10). The aim of this present study was to analyse the effect of experimentally induced changes of thyroid status, with or without acute administration of T₃ and RA, on the properties of RARs and TRs. We also measured the activities of MDH and ADH in the liver of controls and treated animals.
Materials and methods

Experimental design

Official French regulations 87848 and 03195 for the care and use of laboratory animals were followed. Male Wistar rats were obtained from IFFA Credo (L’Arbresle, France). Rats were housed four to a cage in an air-conditioned room of mean temperature 21°C and with a photoperiod that followed the seasonal pattern, varying from 12 to 13 h of light per day during the experiments. Rats had free access to a semi-synthetic diet prepared for us by the “Atelier de préparation d’aliments expérimentaux”, INRA, Jouy en Josas, France. The diet contained (% dry wt): 18% vitamin-free casein, 30.5% sucrose, 2.5% peanut oil, 2.5% rape-seed oil, 2% cellulose, 40% maize starch, 3.5% salt mixture, 1% vitamin Diet Fortification Mixture. This chow contained precisely 1.66 mg of iodide (as KI), and 1.5 mg of retinol (as retinyl palmitate) per kilogram according to the recommendations on the feeding conditions of laboratory animals (rats and mice) (11). After 1 week of adaptation the rats were divided randomly into three groups designated as hypothyroidism, hyperthyroidism and euthyroidism.

Hypothyroidism experiment (38 rats). Eight rats served as controls, while 30 rats received propylthiouracil (6-n-propyl-2-thiouracil (PTU); Sigma, no. P 3755) in their drinking water (0.05% w/v) for 21 days (12). Then, these 30 rats were divided into four subgroups: eight rats were maintained as hypothyroid rats; eight rats were injected intraperitoneally (ip) with triiodothyronine (3,3’,5’-triiodo-L-thyronine; Sigma, no. T 2752) in 0.05 mol/l NaOH at a dose of 25 μg/100 g, twice in the 24 h before sacrifice; seven rats were treated by intragastric intubation with retinoic acid (all-trans retinoic acid (RA); Sigma, no. R 2625) in arachis oil at a dose of 25 μg/100 g, twice in the 24 h before sacrifice; seven rats received T3 and RA as described above.

Hyperthyroidism experiment (23 rats). Eight rats served as controls with 15 rats were injected ip with T3 at a dose of 25 μg/100 g, twice daily in the 24 h before sacrifice; seven rats served as hyperthyroid rats; eight rats were administered by intragastric intubation with RA at a dose of 250 μg/100 g, twice in the 24 h before sacrifice.

Euthyroidism experiment (16 rats). Eight rats served as controls; eight rats were administered with RA as described above.

Rats were killed by decapitation at 09.00 h. Blood was collected and the livers rapidly excised. Portions of liver were immediately used for preparation of nuclear and cytosolic fractions.

Hormone binding

Isolation of liver nuclei. All tissue fractionations were carried out at 4°C. Nuclei were prepared according to the method of De Groot and Torresani (13). An aliquot of liver was homogenized in 0.32 mol/l sucrose plus 1 mmol/l MgCl2 (0.32 SM), filtered through cheesecloth and centrifuged at 1000g for 10 min. The crude pellet was washed once and then centrifuged through a layer of sucrose (2.2 mol/l sucrose plus 1 mmol/l MgCl2) at 100 000g for 60 min. The nuclear pellet was resuspended gently in 0.32 SM plus 0.25% (v/v) Triton X-100, centrifuged at 1000g for 10 min and washed once with 0.32 SM.

Triiodothyronine binding. The final nuclear pellet derived from 2 g of liver was resuspended gently in 2.66 ml of TKEM (20 mmol/l TRIS·HCl, 0.4 mol/l KCl, 2 mmol/l EDTA and 1 mmol/l MgCl2; pH 7.9, 25°C). After 30 min at 0°C with frequent pipetting of the suspension, the nuclear residue was pelleted by centrifugation at 100 000g for 30 min. The supernatant, which contained nuclear proteins, was used for assay of T3 binding (14).

Incubations of nuclear proteins were performed in 0.2 ml of TKEM containing 50 μg of proteins and 0.006–0.12 pmol [125I]T3 for 3 h at 20°C. Because fresh liver tissue was used for obtaining nuclear proteins, dithiothreitol (DTT) has not been added in the incubation medium. Indeed, if DTT is known to enhance T3 binding it may minimize the differences of affinity between the experimental groups of rats. Thus, the affinities measured are lower than that usually reported. The binding reaction was stopped by the addition of 1.8 ml of an ice-cold Dowex IX-8-400 resin (Sigma, St Louis, MO) suspension in TKEM (40 g/l). After mixing, the resin was sedimented by centrifugation (1000g, 5 min).

Estimation of protein-bound T3 was made by measuring radioactivity in an aliquot of the supernatant. Non-specific T3 binding was determined by incubation in the presence of a 1000-fold excess of unlabelled T3. All incubations were performed in duplicate. Saturation curves and Scatchard analysis were performed using final concentrations of [125I]T3 in the incubation medium ranging from 0.03 to 0.6 nmol/l.

Retinol binding. Owing to the sensitivity of RA to numerous physicochemical factors (particularly to light and oxygen) and its rapid degradation by enzymes contained in tissue extracts, a synthetic analogue of RA was used as ligand. This one, the 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl) benzoic acid (CD367), was synthesized and tritiated in the Centre International de Recherche Dermatologique (CIRD Galdema, Sophia Antipolis, Valbonne, France). CD367 behaves as a high-affinity ligand that binds the three types of RARs (RAR-α, RAR-β and RAR-γ).
(15). Previous experiments performed in our laboratory have shown that CD37 can be used validly to study the binding of RAR in rat liver (16).

To obtain RAR, the nuclei were washed three times with binding buffer (10 mMol/l HEPES, 1.5 mMol/l MgCl$_2$, 10 mMol/l KCl, pH 7.9) and then submitted to a DNase I (Sigma no. D 4527) digestion for 30 min at room temperature, followed by a high salt extraction (0.5 mMol/l NaCl). The nuclear extract then was obtained by centrifugation at 12 000 rpm for 5 min. The measurement was performed according to Daly et al. (17). Aliquots of nuclear extract (96 µl) were mixed with 4 µl of increasing concentrations (0.0125–0.125 µMol/l in dimethyl sulphoxide) of CD37 (52.8 Ci/mmol). After 1 h of incubation at 4°C, 50 µl of the incubation mixture was submitted to a high-performance size-exclusion chromatography separation on a TSK gel G3000SW column (300×7.5 mm, Tohso Haas Stuttgart, Germany) and eluted with 0.3 mMol/l K$_2$PO$_4$ (pH 7.8) at a flow rate of 0.5 ml/min. The column was calibrated with a mixture of human albumin (67 kD), egg albumin (45 kD) and horse myoglobin (16.8 kD). Fractions of 0.2 ml were collected and counted in a liquid scintillation counter using Ready Value Cocktail (Beckman) as the scintillation liquid. Radioactive counts obtained in fractions containing the RAR–[1$^3$H]CD37 complex were added and expressed as fmol bound labelled ligand/mg proteins. Non-specific binding was determined by incubation in the presence of 1000-fold excess of unlabelled CD37.

Scatchard analysis. Scatchard curves were drawn using linear regression analysis of the data. The slope of the straight line gave the apparent affinity constant and the intercept of the slope with the abscissa represented the maximum binding capacity, i.e. the maximal concentration of binding sites.

### Assays

Activity of ADH (EC 1.1.1.1) was measured according to Smith et al. (18). l-Malate dehydrogenase NADP oxido-reductase (decarboxylating) (EC 1.1.1.40, MDH) was determined by the method described by Wise and Ball (19). Proteins were measured according to the method of Bradford (20) using a Bio-Rad protein assay (Bio-Rad Laboratories, Munich, Germany). Serum vitamin A was assayed by high-performance liquid chromatography (21). Serum T$_3$ was assayed by a radioimmunoassay using a T$_3$-Amerlex-M RIA kit (Kodak Clinical Diagnostics, France).

### Statistical analysis

Values are given as means ± SEM. The statistical significance of differences between means was calculated by analysis of variance followed by Tukey's multiple-range post hoc test (K = 0.05) using Minitab Statistical Software (USA).

### Results

#### Interactions of RA and T$_3$ on RARs and TRs

The results are summarized in Table 1.

**Hypothyroidism experiment.** In rats made hypothyroid by PTU treatment (T$_3$, serum level: 0.38 ± 0.01 and 0.65 ± 0.03 µg/l in hypothyroid and control rats, respectively), no change was observed in the maximal binding capacity (C$_{max}$) or the affinity of nuclear TRs (Fig. 1A). In these animals the administration of either T$_3$ or RA had no significant effect on the properties of TRs. In contrast the simultaneous administration of T$_3$ and RA induced a 48% increase of the C$_{max}$ and a 40% decrease of the apparent affinity of these receptors.

| Table 1. Binding characteristics of rat hepatic nuclear triiodothyronine receptors (TRs) and retinoic acid receptors (RARs) according to thyroid status and either with or without retinoic acid treatment.|
|-----------------|-----------------|-----------------|-----------------|
| Hormonal state of animals | TRs | RARs |
| | | | |
| | C$_{max}$ (fmol/mg protein) | Affinity (l/nmol) | C$_{max}$ (fmol/mg protein) | Affinity (l/nmol) |
| Hypothyroidism | | | |
| Control (8) | 360 ± 20$^b$ | 4.45 ± 0.34$^{bc}$ | 711 ± 51$^b$ | 1.26 ± 0.0$^b$ |
| Hypothyroid (8) | 380 ± 16$^b$ | 4.85 ± 0.25$^{bc}$ | 477 ± 30$^c$ | 1.15 ± 0.05$^b$ |
| Hypothyroid + T$_3$ (8) | 442 ± 24$^b$ | 3.52 ± 0.29$^{bc}$ | 855 ± 48$^bd$ | 0.36 ± 0.07$^c$ |
| Hypothyroid + RA (7) | 353 ± 27$^b$ | 4.25 ± 0.30$^{bc}$ | 693 ± 59$^b$ | 1.14 ± 0.06$^b$ |
| Hypothyroid + T$_3$ + RA (7) | 563 ± 30$^c$ | 2.90 ± 0.23$^d$ | 1012 ± 46$^d$ | 0.56 ± 0.05$^c$ |
| Euthyroidism | | | |
| Control (8) | 357 ± 15$^b$ | 4.34 ± 0.13$^b$ | 557 ± 34$^b$ | 0.95 ± 0.08$^b$ |
| Control + RA (8) | 388 ± 17$^b$ | 4.00 ± 0.26$^b$ | 315 ± 40$^d$ | 1.85 ± 0.11$^c$ |
| Hyperthyroidism | | | |
| Control (8) | 316 ± 22$^b$ | 5.45 ± 0.35$^b$ | 543 ± 46$^b$ | 0.98 ± 0.10$^b$ |
| Hyperthyroid (7) | 531 ± 18$^b$ | 3.95 ± 0.29$^b$ | 519 ± 45$^b$ | 0.85 ± 0.07$^b$ |
| Hyperthyroid + RA (8) | 416 ± 22$^d$ | 4.20 ± 0.29$^c$ | 329 ± 14$^c$ | 2.32 ± 0.35$^c$ |

$^a$ Values are means ± SEM, with number of rats in parentheses. For each experiment the values are significantly different (p < 0.05) when the superscript letters are different.
Hypothyroidism induced a significant decrease (33%) in the C\text{max} but did not affect affinity of RARs (Fig. 1B). In these hypothyroid animals the administration of RA or T\textsubscript{3} or RA + T\textsubscript{3} induced an increase in the C\text{max} of RARs of, respectively, 45, 79 and 112%. Moreover, after administration of either T\textsubscript{3} alone or T\textsubscript{3} + RA a decreased affinity of these receptors was observed, while the administration of RA alone was without significant effect.

Hyperthyroidism experiment. In rats made hyperthyroid by T\textsubscript{3} treatment (T\textsubscript{3} serum level >50 and 0.71 ± 0.05 μg/l in hyperthyroid and control rats, respectively), an increased C\text{max} and a decreased apparent affinity of TRs were observed (Fig. 1C). After treatment of these animals with RA the C\text{max} of TRs was decreased relative to hyperthyroid rats (but remained greater than in control rats) while the affinity was not affected.

In hyperthyroid rats there was no change in the properties of RARs. The administration of RA to these animals induced a decrease in the C\text{max} and an increase in the apparent affinity of RARs (Fig. 1D).
Table 2. Malate dehydrogenase (MDH) and alcohol dehydrogenase (ADH) activities in liver of rats according to thyroid status and either with or without retinoic acid treatment.

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<tr>
<td>MDH (nmol NADPH·min⁻¹·mg⁻¹ protein)</td>
<td>55 ± 7ᵇ</td>
<td>39 ± 7ᵇ</td>
<td>56 ± 9ᵇ</td>
<td>37 ± 10ᵇ</td>
<td>34 ± 6ᵇ</td>
<td>49 ± 4ᵃ</td>
<td>45 ± 4ᵃ</td>
<td>48 ± 6ᵃ</td>
<td>124 ± 6ᵇ</td>
</tr>
<tr>
<td>ADH (nmol NADH·min⁻¹·mg⁻¹ protein)</td>
<td>29 ± 2ᵇ</td>
<td>46 ± 3ᵇ</td>
<td>45 ± 1ᵇ</td>
<td>51 ± 2ᵇ</td>
<td>42 ± 1ᵇ</td>
<td>36 ± 2ᵃ</td>
<td>28 ± 1ᵇ</td>
<td>28 ± 2ᵇ</td>
<td>17 ± 2ᵇ</td>
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Values are means ± SEM, with number of rats in parentheses. For each experiment, values with the same superscript letter are not significantly different from each other.

**Euthyroidism experiment.** In this experiment the administration of RA in control rats was without apparent effect on TRs but the C_{max} of RARs was decreased while their affinity constant was increased.

**Effect of T₃ and RA on related enzymatic activities**

The results are summarized in Table 2.

**Hypothyroidism experiment.** In hypothyroid rats the MDH activity was decreased and this activity was restored completely by T₃ administration. Retinoic acid treatment alone was without effect. Furthermore, the effect of T₃ was prevented when T₃ and RA were administered together.

Hypothyroidism induced an increased activity of hepatic ADH, which was not corrected by administration of T₃, RA or T₃ + RA.

**Hyperthyroidism experiment.** In hyperthyroid rats MDH activity was significantly greater than in control rats and RA treatment was without apparent effect on this increase of enzymatic activity.

Hyperthyroidism induced a decrease of ADH activity and RA administration did not modify this change.

**Euthyroidism experiment.** After RA administration to control rats the activity of MDH was not affected and that of ADH was decreased.

**Discussion**

**In hyperthyroid rats the administration of RA decreases the binding capacity of TRs and RARs**

In agreement with Nakamura et al. (22), an increased binding capacity of nuclear TRs in liver was observed after T₃ treatment. Such an observation cannot be considered the result of a simple upregulation of the TR gene expression because it has been shown that the messenger RNA of TR-β (the predominant form of TR in rat liver) is unaffected by T₃ levels (23). Moreover, an extensive study by Hodin et al. (24) on tissue-specific change in TR isoform mRNAs has demonstrated that the level of c-erbA β₁ in rat liver is unaffected by thyroid status. Thus it is possible that the increased binding capacity was the result of biochemical events occurring at a post-transcriptional level and possibly from changes in the functional properties of receptors. After RA administration to hyperthyroid rats the C_{max} of TRs was restored partly. Such a result can be related to the amelioration of symptoms in hyperthyroid patients treated with large doses of retinol (25) and that vitamin A treatment decreases the severity of experimental thyroiditis in guinea pigs and rats (26).

Hyperthyroidism did not affect the binding capacity of RARs, a result in agreement with that of Kato et al. (27) who showed the thyroid hormone has no effect on the level of RAR transcripts. In hyperthyroid rats a 1-day treatment with RA induced a decrease of the binding capacity of RARs. The effect of RA administration in hyperthyroid rats was similar to that observed in euthyroid rats treated in the same way. Such a result was unexpected because an upregulation of RARs by retinoids has been observed by some workers. Thus, Haq et al. (28) and Kato et al. (27) have shown that RA increases the level of the RAR-β transcripts in the liver of retinol-deficient rats (this transcript is the predominant form of RARs in rat liver). Moreover, in our laboratory Audouin-Chevallier et al. (16) have reported an increased binding capacity of RARs in the liver of rats fed on a vitamin A-overloaded diet for 7 weeks. The discrepancy between these observations and our results may be related to the very different experimental conditions. Thus, in the experiments reported above the vitamin A status of rats was altered markedly while in our study the vitamin A status of the hyperthyroid rats was not affected (the retinol serum level was...
Fig. 2. Changes in binding capacity of triiodothyronine (T3) receptors (TRs) and retinoic acid (RA) receptors (RARs) in the liver of rats made hypothyroid or hyperthyroid after the animals were treated with T3, RA or T3 + RA. The values of Cmax represent the percentages of Cmax measured in control rats in each experiment. *Significantly different from corresponding controls (Tukey test).

448 ± 11 and 447 ± 27 µg/l, respectively, in control and hyperthyroid rats).

In hypothyroid rats the administration of RA increases the binding capacity of RARs but not that of TRs

The binding capacity of TRs was not affected in the liver of rats rendered hypothyroid by PTU administration for 20 days. A similar result was reported by Bernal et al. (29) who observed that thyroidectomy does not affect the binding capacity of these nuclear receptors. Retinoic acid administration to hypothyroid rats did not affect the binding capacity of TRs.

In contrast, a decreased binding capacity of RARs was observed in hypothyroid rats. Such an observation can be considered, at least in part, the results of the change of vitamin A status of animals induced by hypothyroidism, because in this experimental condition the serum level of retinol was decreased by 30% relative to the control value (594 ± 22 and 417 ± 37 µg/l in control and hypothyroid rats, respectively). Indeed, it is known that a vitamin A-deficient status brought about a reduced Cmax of RARs (16, 27, 28). In our experiments the administration of RA to hypothyroid rats led to an increased capacity of RARs, which can be considered the result of an upregulation of RARs by RA.

In hypothyroid rats the simultaneous administration of T3 + RA induces an increase in the binding capacity of both TRs and RARs

Interestingly, while the administration of T3 or RA was without effect on the properties of TRs, the simultaneous administration of T3 and RA induced an increase of the binding capacity of these receptors. Moreover, the effect of such a simultaneous administration on the binding capacity of RARs was greater than that of either T3 or RA alone. Thus, RA potentiated the effect of T3 on the Cmax of RARs. These results can be related to recent data showing a ligand-dependent conformational change in TRs and RARs (30). Also, it has been shown that heterodimerization between TR or RAR and RXR enhances the ligand-dependent change and that dual hormone treatment (by T3 and 9-cis RA) intensifies this enhancement (30).

The apparent affinities of TRs and RARs are similarly modified in the hypothyroidism experiment and differently modified in the hyperthyroidism experiment

Hypothyroidism itself had no effect on the affinity constant of TRs and RARs and the administration of T3 (alone or with RA) to hypothyroid rats induced a decrease of affinity constant for both TRs and RARs. The
similar changes that occurred in both TRs and RARs constitute an argument in favour of a true biological action of $T_3$ on the affinity of these receptors. Thus, the lowered affinity of the receptors cannot be considered solely as an artifact resulting from an increased amount of endogenous $T_3$ as suggested by Nakamura et al. for TRs (22).

In contrast, very different results were obtained when the affinity constants of TRs and RARs were measured in hyperthyroid rats. This may be the result of the multiplicity of factors that determines the functionality of these receptors. It must be remembered that these functional receptors are protein dimers and consequently the affinity of these dimers for ligands may depend on the monomers involved in the heterodimerization (RARs or TRs, and RXRs). In addition, the activity of TRs is known to be altered by phosphorylation (31, 32) but no information is available concerning a possible phosphorylation of RARs. In fact, phosphorylation of TRs is increased by PKC (33), which in turn is modulated by several factors, including retinoids and thyroid hormone. Indeed, RA activates PKC (7, 34, 35) and hypothroid rats have increased levels of immuno-detectable PKC, which can be decreased by treatment with thyroxine (36).

**Binding capacity of receptors and resultant enzymatic activities**

The changes of MDH activity observed in hypothyroid and hyperthyroid rats are in agreement with the well-known effect of $T_3$ status on this enzymatic activity. The administration of RA was without effect on this enzymatic activity and, curiously, the administration of RA to hypothyroid rats prevented the effect of $T_3$ administered simultaneously. It has been shown previously (37) that overexpression of one steroid receptor can interfere with the expression of another steroid receptor. In our study we showed that there was a marked increase in RARs after administration of $T_3 + RA$ to hypothyroid rats, so that this receptor could be considered as overexpressed and subsequently repressing of TRs.

The ADH activity increased in hypothyroid rats and decreased in hyperthyroid animals. This result is in agreement with that of Mezey and Potter (38) showing an increase of ADH activity in the liver of hypothyroid rats and a reduction of this enzymatic activity in hyperthyroid animals. Recently, Harding and Duester (39) have demonstrated that the normal functioning of the ADH-3 RA response element is abolished by TR in the presence of $T_3$. The administration of RA to hypothyroid or hyperthyroid rats was without apparent effect on ADH activity and the administration of the retinoid to euthyroid rats induced a decreased activity. This result does not support the idea of an upregulation of ADH activity by RA as suggested by Duester et al. (7). In further studies it will be interesting to investigate the activity of "tissue transglutaminase", whose expression is induced by RA in hepatocytes (40) and which is possibly a better index of RARs expression than ADH activity.

**Conclusion**

These results show a modulation of the binding properties of TRs and RARs by RA, which was dependent on the thyroidal status. Such a modulation constitutes an element of the complex network of interactions between thyroid hormones, retinoids and their receptors in the signalling pathways. It can be hypothesized that this modulation is the result of various mechanisms that are not mutually exclusive. One of them could be a direct ligand-dependent conformational change. Another might be a ligand-dependent change in the level of expression of monomeric receptors and of the subsequently formed dimers.

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