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Retinoids Modulate the Binding Capacity of the Glucocorticoid Receptor and its Translocation from Cytosol to Nucleus in Liver Cells

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The binding capacity (C_{max}) of the glucocorticoid hormone receptor (GR) was affected by vitamin A status in rat liver. In rats fed on a vitamin A-overloaded diet as well as in rats administered with retinoic acid (RA) there was an increased ratio C_{max} of nuclear GR (expressed as fmol/mg liver): C_{max} of cytosolic GR (expressed as fmol/mg liver) while in rats fed on a vitamin A-deficient diet this ratio was decreased. These results suggested that an increased amount of RA, resulting from either metabolization of an increased amount of dietary retinol or RA administration, enhanced the translocation of GR from the cytosolic compartment to the nuclear compartment. Moreover such an increased amount of RA could also induce the observed decreased C_{max} of the total GR that we observed. These observations were similar to the well known effects of dexamethasone administration on the properties of GR. It is probable that RA, similarly to dexamethasone treatment, induces a dissociation of the tetrameric form of the cytosolic GR and thus enhances translocation of the monomeric form from cytosol to nucleus and also resulting in an increased proteolytic degradation of the receptor.

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INTRODUCTION

Glucocorticoid hormones are active in regulating metabolic pathways and in promoting the development of various organs as well as the growth of many cell types *in vitro*. These effects are mediated by a soluble protein in target cells called the glucocorticoid receptor (GR). GR belongs to a large "superfamily" of receptors that includes steroid, thyroid and retinoic acid (RA) receptors [1, 2]. GR was found to be mainly localized in the cytoplasm of target cells not previously exposed to the hormone. Following hormone stimulation, GR translocates to the nucleus and exerts its control of gene expression either by acting on the gene response element as transcription factor or by interacting with other trans-acting factors such as the AP_1 transcription factor [3, 4]. Moreover it is known that the receptors of the superfamily are submitted to either homologous or heterologous regulation processes (regulation of receptor by a ligand which is or which is not its own ligand, respectively). It was previously shown in our laboratory that RA administered either directly to rats or resulting from the metabolization of dietary retinol modifies the binding properties of the thyroid hormone receptor in rat liver [5–8]. In contrast, little is known about the effect of vitamin A status on the binding properties of GR. Using cultured cells it was shown [9] that in retinol-treated cells there was decreased dexamethasone binding. The aim of this study was thus to analyse the effect of induced changes of vitamin A status on the binding properties of GR in rat liver while at the same time taking in account the subcellular localization of the receptor. We have thus investigated separately the binding properties of cytosolic GR (GRc) and nuclear GR (GRn), which is the GR directly involved in the regulation of gene expression. Moreover we have attempted to provide an explanation for the change of binding capacity of the total GR of liver cells (GRs) by assaying mRNA levels for this receptor.

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EXPERIMENTAL

Animals

Official French regulations for the care and use of laboratory animals were followed. Male Wistar rats were obtained from IFFA CREDO (L'Arbresle, France) and were housed in groups of four in cages in an air-conditioned room with a mean temperature of 21°C with a photoperiod which followed the seasonal pattern and which varied from 12 to 13 h of light per day during the vitamin A and steroid experiments.

Vitamin A experiment. Thirty two weanling rats were randomly divided into three groups designated as vitamin A-deficient (−A rats, $n = 16$), vitamin A-overload (+AA rats, $n = 8$) and vitamin A-adequate or control (C_1 rats, $n = 8$). The pelleted diets were prepared to our specification (vitamin A-adequate diet according to the recommendations on the feeding conditions of the laboratory animals [10]) by the "Atelier de préparation d'aliments expérimentaux", Centre de recherche INRA, Jouy en Josas, France. The −A rats were fed a vitamin A-free diet. The C_1 and +AA rats were fed the same diet supplemented with 2.7 and 110 mg all-trans retinoyl palmitate (ROCHE, Paris, France) per kg of diet, respectively. Diet and water were freely available. Body weights were recorded daily throughout the experiment. The daily food intake was calculated, and during the experiment the food intake of the deficient and overloaded rats was similar to that of the normal animals. The +AA rats were studied after 7 weeks of exposure to the diet. Measurements on the −A rats were performed when the animals were at the beginning of their weight plateau and without apparent clinical symptoms (approx. after 7–8 weeks on the vitamin A-deficient diet). Some −A rats were administered with a single dose of 100 μg/rat of all-trans retinoic acid (RA; R2625, Sigma Chemical Company, St Louis, MO) by stomach tube, and studied 12 h after administration (−A + RA rats).

RA administration to control rats. Eight rats weighing 240–250 g, on a vitamin A-adequate diet served as controls (C_2 rats) while 8 other rats received 2 μg/g body weight of RA in arachis oil by stomach tube at 09.00 a.m. and 09.00 p.m. the day before sacrifice (C_2 + RA rats).

Hypocorticosteroid experiment. Twenty four rats weighing 200–250 g were used. Sixteen were adrenalectomized under sodium pentobarbital anesthesia and subsequently received 0.9% NaCl in their drinking water and a vitamin A-adequate diet while 8 were sham-operated controls. Absence of identifiable adrenal tissue was verified in each animal by post-mortem examination. Seven days after surgery, 8 adrenalectomized animals were administered with 2 μg/g b.wt. of RA in arachis oil by stomach tube at 09.00 a.m. and 09.00 p.m. the day before sacrifice (Adx + RA rats). Adrenalectomized rats not treated by RA (Adx rat) and sham-operated rats (C_3 rats) received vehicle only.

Hypercorticosteroid experiment. Twenty four rats weighing 200–250 g were used. Eight rats served as controls while 16 rats were administered with dexamethasone 21-phosphate (Dx) (D1159, Sigma Chemical Company, St Louis, MO) in NaCl 0.9% at a dose of 2 μg/g b.wt. twice daily 3 days before sacrifice. Among these hypersteroid rats 8 received 2 μg/g b.wt. of RA in arachis oil by stomach tube at 09.00 a.m. and 09.00 p.m. the day before sacrifice (Dx + RA rats). Hypercorticosteroid not treated by RA (Dx rats) and controls rats (C_4 rats) received vehicles only.

Rats were killed by decapitation at 09.00 a.m. and the liver rapidly excised. Portions of liver were immediately used for preparation of nuclei and cytosol fractions, the remainder being frozen in liquid nitrogen and stored at −80°C for subsequent analysis. All manipulations were carried out at 4°C.

Binding studies

Cytosol preparation. Rat liver was homogenized in 3 vol. ice-cold buffer A (Tris–HCl (10 mmol/l), dithiothreitol (DTT; 2 mmol/l), EDTA (1.5 mmol/l), sodium molybdate (20 mmol/l), 100 g/l glycerol, pH 7.4) with an Ultra-Turrax. Molybdate has a stabilizing effect on the non-activated glucocorticoid receptor complex with its hsp90, preexisting in cytosolic preparation. The homogenate was centrifuged at 20,000 *g* for 20 min, followed by 105,000 *g* for 1 h and the resulting supernatant, referred to as "cytosol", was used in the binding experiments [11].

Nuclear preparation. An aliquot of liver was homogenized in 0.32 SM (0.32 mol/l sucrose plus 1 mmol/l $MgCl_2$) filtered through cheesecloth and centrifuged at 1000 *g* for 10 min. The crude pellet was washed once and then centrifuged through a layer of sucrose (2.2 mol/l sucrose plus 1 mol/l $MgCl_2$) at 100,000 *g* for 60 min. The nuclear pellet was gently resuspended in 0.32 SM plus 0.25% (v/v) Triton X-100, centrifuged at 1000 *g* for 10 min and washed once with 0.32 SM [12].

Cytosolic GR binding. Cytosol was diluted with buffer A to give a final concentration of 5 g protein/l. Aliquots (0.5 ml), in duplicate, of this solution were incubated overnight (16–20 h) at 4°C with a range of labelled [^{3}H]dexamethasone (40 Ci/mmol; Amersham, Les Ulis, France), concentrations (1–15 nmol/l), either alone, or in the presence of a 500-fold excess of unlabelled dexamethasone (D1756, Sigma Chemical Company, St Louis, MO), to determine non-specific binding [11]. This long incubation period was necessary to reach binding equilibrium at low concentrations [13]. Bound and free hormone fractions were separated by incubation with an equal volume (v/v) of DCC suspension (50 g/l activated charcoal, 5 g/l dextran in buffer A) for 10 min and centrifugation at 1500 *g* for 10 min. Aliquots of supernatant were counted in 4 ml Ready Value liquid scintillation cocktail (Beckman,

Marseille, France) in a Beckman LS 6000 SC scintillation counter.

Nuclear GR binding. This study was performed according to Kaufman and Shaper [14] with slight modifications. Nuclei were resuspended in STM buffer (250 mmol/l sucrose, 50 mmol/l Tris–HCl (pH 7.4 at 4°C), 5 mmol/l $MgSO_4$). Duplicate 0.1 ml aliquots of this suspension were incubated overnight (16–20 h) at 4°C with a range of labeled [^{3}H]dexamethasone, concentrations (10–100 mmol/l) either alone, or in the presence of 1000-fold excess of unlabeled dexamethasone to determine non-specific binding. After incubation, the samples were diluted with 2 ml STM buffer and sedimented at 1300 *g* for 15 min. The pellets were resuspended and washed three times with 3 ml of STM buffer to remove unbound hormone. After the final sedimentation samples were extracted with 1 ml ethanol at 22°C for a minimum of 30 min, cooled to 4°C, and sedimented at 1300 *g* for 15 min. Aliquots of supernatant were counted with 4 ml Ready Value liquid scintillation cocktail (Beckman, Marseille, France) in a Beckman LS 6000 SC scintillation counter.

Quantification of mRNA

The absolute values of GR mRNAs cannot be determined directly, but a proportion can be deduced by comparing them with the β-actin internal standard simultaneously reverse-transcribed and amplified in the same test tube.

Extraction of RNA was performed according to Chomczinski & Sacchi [15] (modified). Rat liver (300 mg) was homogenized with 6 ml of extraction buffer (3:1; 5.3 M guanidium thiocyanate, 0.2 M Tris–HCl pH 7.5, 0.04 M EDTA/solution DTT—*N*-lauryl sarcosin 2%), and subsequently total RNA was extracted from this homogenate with an equal volume of phenol/chloroform/isoamyl alcohol (49:49:2).

Oligonucleotide primers used for PCR were synthesized using an Applied Biosystem Model 381A DNA synthetizer. The positions and sequences of the different primers are summarized in Table 1. The position of the primer for GR mRNA was chosen according to the sequence of Miesfield *et al.* [16] and those for β-actin mRNA were chosen according to the sequence of Nudel *et al.* [17].

Preparation of cDNA. 110 μg of total mRNA, 550 ng of primer A2 and 550 ng of primer G2 were used for the reverse transcription in the presence of 12.5 μl of reaction buffer 5 × (250 mM Tris–HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT), 440 U of Moloney Murine Leukemia Virus reverse transcriptase, 88 U RNase inhibitor, 55 U DNase and 120 μM of each dNTP in a total volume of 55 μl. Synthesized cDNA was then amplified by the polymerase chain reaction (PCR) technique using Taq polymerase [18].

PCR analysis. 15 μl of cDNA was used for amplification performed in a Perkin Elmer/Cetus thermocycler. The reaction mixture (180 μl) contained 600 ng of each primer A2, G2, A1, G1, 10 mM Tris–HCl pH 8.5, 50 mM KCl, 2 mM $MgCl_2$, 10 mg/l gelatin, 0.2 mM of each dNTP, 50 μCi deoxycytosine triphosphate (sp. act., 370 MBq/ml, Amersham) and 1.25 U Taq polymerase. The reaction was carried out for a total of 34 cycles. The cycle times were as follows: denaturation, 1 min at 95°C; annealing, 1 min at 60°C; primer extension, 2 min at 72°C.

For quantitative analysis of PCR products, 8 μl of PCR reaction were sampled after each (from 10th to 29th) amplification cycle and then at the last [19], and the coamplified fragments were separated by electrophoresis on a 10% acrylamide gel. The incorporated radioactivity was visualized by autoradiography, the bands were excised from the gel and quantified by scintillation counting.

Scatchard analysis

Scatchard curves [20] were drawn using a linear regression analysis of the data (SigmaPlot Scientific Graphing System1 4.02, JANDEL Corporation). The slope of the straight line gave the affinity constant (K_a) and the intercept of the slope with the abscissa represented the maximum binding capacity (C_{max}), i.e. the maximal concentration of binding sites.

Statistical analysis

Values are given as means ± SEM. The statistical significance of differences between means were calculated by analysis of variance followed by Tukey

Table 1. *Sequences of oligonucleotide primers and size of amplified fragments*

Primers		Sequences 5′ → 3′	Complementary sites	Size of amplified fragments (bp)
β-actin*	A1	AGGATGCAGAAGGAGATTACTGCC	2814–2837	222
	A2	GTAAAACGCAGCTCAGTAACAGTCC	3159–3135	
GR†	G1	TGAGACCAGATGTAAGCTCTCCTC	1321–1344	167
	G2	AATTGTGCTGTCCTTCCACTGCTC	1488–1465	

*From rat cytoplasmic β-actin gene according to the sequence of Nudel *et al.* [17].

†From rat GR cDNA according to the sequence of Miesfield *et al.* [16].

Primers A1 and A2 being chosen in two different exons, the size of the PCR products allowed us to ensure that there was no genomic DNA origin in the amplified fragment.

Table 2. Effect of the dietary level of vitamin A on the binding properties of cytosolic and nuclear GR in rat liver

	Cytosolic GR		Nuclear GR	
	C_{max} fmol/mg liver (fmol/mg prot.)	Affinity K_a (nM^{-1})	C_{max} fmol/mg liver (fmol/mg prot.)	Affinity K_a (nM^{-1})
Control diet	35.81 ± 2.25^a (438 ± 27)	0.78 ± 0.07^a	1.07 ± 0.06^a (633 ± 45)	0.015 ± 0.003^a
Vitamin A-deficient diet	46.32 ± 1.60^b (620 ± 22)	0.58 ± 0.06^b	0.38 ± 0.03^b (192 ± 11)	0.010 ± 0.002^a
Vitamin A-overloaded diet	35.69 ± 1.90^a (420 ± 23)	0.81 ± 0.03^a	1.55 ± 0.07^c (831 ± 44)	0.015 ± 0.002^a

Each value represents the mean ± SEM of 8 animals; [a,b,c] within columns mean values with different superscript letters were significantly different using ANOVA followed by Tukey's test.

multiple range *post hoc* test ($K = 0.05$) using Minitab Statistical Software (U.S.A.).

RESULTS

The Scatchard analysis enabled a calculation of C_{max} of GRc and GRn as fmol/mg protein. However in order to discuss the effect of retinoids on the level of total GR in liver cell (GRs) it was necessary to know the binding capacity of GRs and such a parameter was obtained by adding the C_{max} of GRc (as fmol/mg liver) and C_{max} of GRn (as fmol/mg liver). Such calculations confirmed that GRc constituted the major part of GR in liver cells since in control rats the binding capacity of GRn only was 1.5–5% (according to the experiments) of the binding capacity of GRs.

Effect of dietary level of vitamin A on the binding properties of cytosolic and nuclear GR

The various measurements (Table 2) we have performed on GRc in control rats showed that C_{max} value was between 35.81 and 45.4 fmol/mg liver (438 and 625 fmol/mg protein) and the K_a value was near 1 nM^{-1}, a result in agreement with numerous authors [21, 22, 11]. Concerning GRn the C_{max} measured was lower than that reported by Kaufmann and Shaper [14] while K_a value was in agreement with these authors.

Vitamin A-deficiency induced an increased C_{max} and a decreased K_a of GRc while a vitamin A-overloaded diet had no effect on the binding properties of this receptor. In contrast, vitamin A-deficiency induced a decreased C_{max} and a vitamin A-overloaded diet induced increased C_{max} of GRn.

Effect of RA administration on the binding properties of cytosolic and nuclear GR according to vitamin A or corticosteroid status

In control rats RA administration had no significant effect on the C_{max} of cytosolic GRc but induced an increased C_{max} of nuclear GRn (Table 3).

In vitamin A-deficient rats, RA induced a decreased C_{max} and an increased K_a of GRc and simultaneously an increased C_{max} of GRn. Theses RA-induced-modifications completely (GRc) or partly (GRn) corrected the effect of vitamin A-deficiency.

RA administration in adrenalectomized rats induced a decreased C_{max} of GRc and an increased C_{max} of GRn and so corrected the effects of adrenalectomy on these parameters.

RA administration in dexamethasone treated rats had no effect on binding parameters of GRc but increased the C_{max} of GRn whose value became similar to that of control rats.

GR mRNA and binding capacity of GRs

In some experiments we have attempted to relate binding capacity of GRs with the level of GR mRNA (Table 4). In vitamin A-deficient rats the level of mRNA was not significantly decreased, while C_{max} of GRs was increased. In contrast a vitamin A-overloaded diet induced an increased level of mRNA while C_{max} of GRs was not affected.

RA administration in adrenalectomized rats induced a decreased C_{max} while the level of of mRNA was slightly affected. RA administration in dexamethasone treated rats had no significant effect either on C_{max} or GR mRNA level.

DISCUSSION

Effect of retinoid status on the distributions of GRs between cytosol and nucleus

It is well known that GR located in cytosol (GRc) constitutes the major part of GRs in cells and thus the modification of the binding capacity of the GRs mainly reflected that of GRc but the knowledge of the relative amount of GRn is crucial since only these latter regulate gene expression by acting as a transcription factor.

Figure 1 shows that when the vitamin A status was changed (by either modifying the level of dietary retinol or RA administration) the ratio GRn:GRc was modified. Thus in the liver of rats on a vitamin A-deficient diet this ratio decreased while this ratio increased in the liver of rats on a vitamin A-overloaded diet as well as in the liver of rats administered with RA. It can be estimated that the level of the dietary retinol

Table 3. Effect of RA administration on the binding properties of cytosolic GR and nuclear GR in rat liver according to vitamin A or corticosteroid status

Experiments	Cytosolic GR		Nuclear GR	
	C_{max} fmol/mg liver (fmol/mg prot.)	Affinity K_a (nM^{-1})	C_{max} fmol/mg liver (fmol/mg prot.)	Affinity K_a (nM^{-1})
Exp. 1				
Control rats	44.91 ± 3.14^a (503 ± 34)	0.85 ± 0.11^a	0.96 ± 0.05^a (644 ± 38)	0.010 ± 0.002^a
Control rats + RA	41.17 ± 3.03^a (494 ± 40)	0.87 ± 0.08^a	1.21 ± 0.05^b (788 ± 41)	0.014 ± 0.003^a
Exp. 2				
Control rats	35.81 ± 2.2^a (438 ± 27)	0.78 ± 0.07^a	1.07 ± 0.06^a (633 ± 45)	0.015 ± 0.001^a
Vitamin A-deficient rats	46.32 ± 1.60^b (620 ± 22)	0.58 ± 0.06^b	0.38 ± 0.03^b (192 ± 11)	0.010 ± 0.002^a
Vitamin A-deficient rats + RA	34.37 ± 1.78^a (439 ± 24)	1.01 ± 0.05^c	0.46 ± 0.02^b (254 ± 18)	0.017 ± 0.003^a
Exp. 3				
Control rats	45.40 ± 2.53^a (625 ± 35)	1.05 ± 0.08^a	1.25 ± 0.08^a (802 ± 45)	0.021 ± 0.004^a
Adrenalectomized rats	55.21 ± 2.45^b (817 ± 36)	1.14 ± 0.09^a	0.29 ± 0.04^b (214 ± 25)	0.010 ± 0.002^b
Adrenalectomized rats + RA	41.63 ± 1.93^a (632 ± 29)	0.84 ± 0.09^a	1.16 ± 0.07^a (778 ± 46)	0.013 ± 0.003^b
Exp. 4				
Control rats	44.91 ± 3.14^a (503 ± 34)	0.85 ± 0.11^a	0.96 ± 0.05^a (644 ± 38)	0.010 ± 0.002^a
Dexamethasone treated rats	11.03 ± 2.72^b (150 ± 36)	0.13 ± 0.09^b	0.61 ± 0.06^b (389 ± 39)	0.018 ± 0.003^a
Dexamethasone treated rats + RA	8.05 ± 1.19^b (103 ± 15)	0.20 ± 0.03^b	0.86 ± 0.05^a (580 ± 26)	0.014 ± 0.002^a

Each value represents the mean ± SEM of 8 animals; [a,b,c] for each experiment and within columns values with the same superscript letter are not significantly different from each other, using ANOVA followed by Tukey's test.

influences the amount of liver RA since RA is a natural metabolite of retinol and particularly that a vitamin A-deficient diet induced a low level of RA. These observations suggested that RA enhanced the translocation of GRs from the cytosol to the nucleus.

For some years the mechanisms involved in the translocation of GRs has been discussed since regulation of this mechanism could constitute a subtle means of regulating gene activity [23, 24]. It is known that the nuclear localization of GR is under control of glucocorticoid hormone [25] and that nuclear localization signals are present in GR [26, 27]. Moreover GR

Table 4. GR mRNA and binding capacity of total GR in some experimental conditions in rat liver

	GR mRNA	C_{max} of GRs (fmol/mg liver)
Control diet	0.110 (0.113–0.107)	36.9 ± 2.29
Vitamin A-deficient diet	0.107 (0.108–0.106)	46.7 ± 1.66
Vitamin A-overloaded diet	0.148 (0.155–0.141)	37.3 ± 2.03)
Control rats	0.111 (0.132–0.087)	46.7 ± 2.61
Adrenalectomized rats	0.158 (0.170–0.146)	55.5 ± 2.46
Adrenalectomized rats + RA	0.139 (0.132–0.145)	42.8 ± 1.98
Control rats	0.120 (0.130–0.110)	45.9 ± 3.10
Dexamethasone treated rats	0.081 (0.083–0.078)	11.6 ± 2.71
Dexamethasone treated rats + RA	0.090 (0.095–0.085)	8.9 ± 1.21

Values of GR mRNA are means of two measurements performed on pools of 4 different rats (the value of each measurement is given in parenthesis).

Values of C_{max} of GRs were obtained by adding C_{max} of cytosolic GR (expressed as fmol/mg liver) and C_{max} of nuclear GR (expressed as fmol/mg liver).

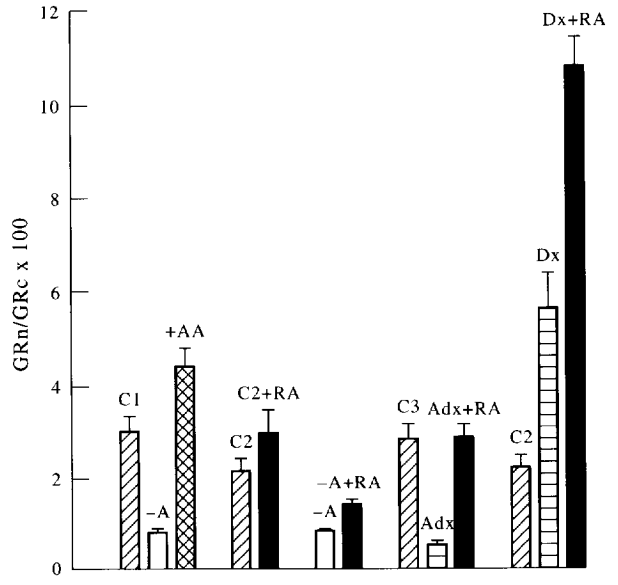

Fig. 1. Effect of vitamin A status and/or RA administration on the ratio binding capacity of nuclear GR (fmol/mg liver): binding capacity of cytosolic GR (fmol/mg liver) in rat liver. C_1, C_2, C_3, C_4, control rats in vitamin A status, retinoic acid administration, hypocorticosteroid, and hypercorticosteroid experiments, respectively; −A, vitamin A-deficient rats; +AA, rats on a vitamin A-overloaded diet; C_2 + RA, control rats administered with RA; −A + RA, vitamin A-deficient rats administered with RA; Adx, adrenalectomized rats; Adx + RA, adrenalectomized rats administered with RA; Dx, dexamethasone treated rats; Dx + RA, dexamethasone treated rats administered with RA.

is a phosphoprotein [28] and phosphorylation of the receptor could influence its nuclear translocation [29]. Some kinases, and among them protein kinase C (PKC), have been suspected to catalyze this phosphorylation [30, 31]. Interestingly, we have previously shown that RA activates PKC activity in rat liver [7]. Thus it can be hypothesized that RA enhanced the phosphorylation of GRc—via an enhanced PKC activity—and subsequently the translocation of GR from the cytosolic to the nuclear compartment of the liver cell.

Also it is known that GR translocates as an activated monomer [32] so that the translocation preliminarily needs a hormone-dependent dissociation of the tetrameric complex which constitutes the cytosolic form of GR [33]. Thus it appears that an experimentally induced increase of RA led to a sequence of biochemical events (dissociation of the cytosolic complex and subsequently increased translocation) similar to that induced by glucocorticoid hormone.

Effect of the retinoid status on the binding capacity of GRs

A vitamin A-overloaded diet had no effect on the binding capacity of GRs in the liver of rats while a vitamin A-deficient diet administered for 7–8 weeks increased this binding capacity (Fig. 2). Values of the binding capacity of GRs measured in rats under both of these different diets could not be correlated with the values of the amount of mRNA of these receptors. Such an observation supports the idea that retinol or more probably its metabolite, RA, modified the binding capacity of GRs mainly at a post-transcriptional level. Such an interpretation is strengthened by the results obtained after acute administration of RA.

The intragastric administration of RA was used to analyse the acute effect of this retinoid on the binding properties of GRs in rats under various experimental conditions including adrenalectomy and dexamethasone treatment. The modifications induced by adrenalectomy or dexamethasone treatment have been reported but won't be discussed because they are in agreement with known data. Indeed, it is established that treatment of rats with dexamethasone induces a down-regulation of the binding capacity of GRs resulting in part from a decreased level of mRNA of these receptors [34, 35]. Also it was observed that adrenalectomy increased the binding capacity of GRs in rat liver [36, 21, 37, 38]. Interestingly, whatever the experimental status of rats, a 1 day treatment by RA was sufficient to induce a decreased binding capacity of GRs.

Our results showed that when retinoid status was modified (by either a vitamin A-deficient diet or RA administration) there was a modulation of the binding properties of GRs. Thus, the level of RA could be considered as determining, at least in part, the binding properties of GRs: when RA was deficient the amount on GRs in liver increased and when RA was administered the amount of GRs decreased. Such a modulation of a receptor by a ligand which is not its own ligand is generally termed as heterologous regulation. There are in the literature few data reporting a heterologous

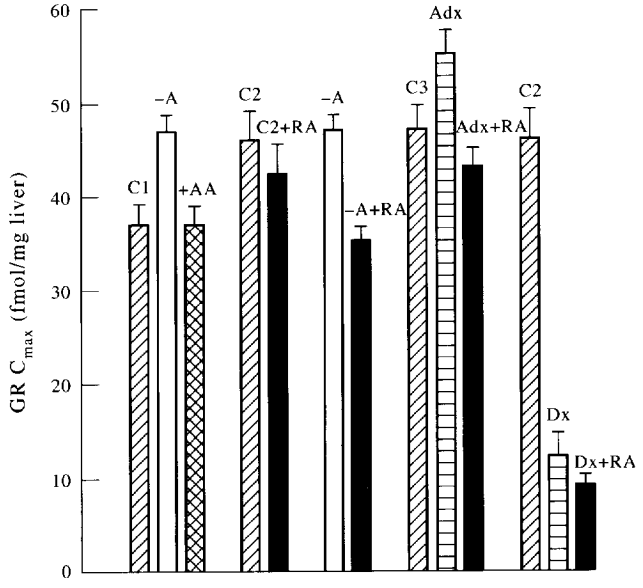

Fig. 2. Effect of vitamin A status and/or RA administration on the binding capacity of total GR in rat liver (the values were obtained by adding C_{max} of cytosolic GR [fmol/mg liver] and C_{max} of nuclear GR [fmol/mg liver]). Abbreviations as in Fig. 1.

regulation of GRs by retinoids and these data, which were only obtained using cell lines, are ambiguous. Thus retinol down-regulates the GRs [9] in cultured T-cells while addition of RA increases 3-fold the binding of dexamethasone in embryonic skin cells [39].

The changes that we have observed in the binding capacity of GRs as a function of the retinoid status can be related to the known changes according to the steroid status. It has been previously noted that activation of GR is needed before its translocation but it is also known that the activated receptor, which is a monomeric form, is more susceptible to proteolytic degradation. The enhanced proteolysis observed after dexamethasone treatment of rats partly explains (another cause being decreased mRNA levels) the low level of GRs in this experimental situation [35]. Thus RA could behave on the turnover of GRs as does dexamethasone and thus induce an increased turnover of the receptor. So the observed heterologous regulation of GRs by RA could be the result of biochemical events occurring at a post-transcriptional level.

These results provide some new evidence on the mechanisms by which retinoids can modulate hormonal status. Furthermore, the present data highlight the multiplicity of factors, and among them nutritional factors, implicated in cellular signalling pathways. Also these results could constitute an experimental argument in favour of the feasability of using nutritional approaches to prevent or correct certain hormonal diseases.

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