Aging decreases the abundance of retinoic acid (RAR) and triiodothyronine (TR) nuclear receptor mRNA in rat brain: effect of the administration of retinoids

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Received 23 April 1997; revised version received 23 June 1997

Abstract Aging is accompanied by troubles resulting from changes in hormonal and nutritional status. Therefore, the abundance of mRNA coding for triiodothyronine (TR) and retinoic acid (RA) nuclear receptors was studied in the brain of young, adult and aged (2.5, 6 and 24 months, respectively) rats. In the brain of aged rats, there was a lower abundance of TR and RAR mRNA and a lower activity of tissue transglutaminase (tTG), an enzyme the gene of which is a target for retinoids. Administration of RA in these rats restored TR and RAR mRNA and the activity of tTG in the brain. The importance of these observations to the function of the aged brain is discussed.

Key words: Aging; Nuclear receptor; RAR; TR; mRNA; Retinoid

1. Introduction

The brain is particularly affected by aging and the neuroanatomical changes observed in aged animals include nerve cell loss, dendritic spines reduction, and modification of synaptic plasticity. Among age-related diseases, some result from modifications in hormonal status such as a decrease in responsiveness to hormones, as well as a deficit of transcription and translation of certain messengers [1]. Aging affects thyroid function inducing, first, a decrease in the rate of hormone degradation and, second, via negative feedback loops, a decrease in the secretory rate of the hormone so that the serum level of triiodothyronine (T_3) is slightly reduced or unaltered [2,3].

Vitamins can also be involved in the development of problems during aging. Indeed, several physiological and biochemical modifications can alter the vitamin status and changes in availability of vitamins can have a drastic effect when these vitamins are able to modulate genic transcription, such as in the case of vitamin A [4,5]. Retinoic acid (RA), which is the active metabolite of vitamin A, and T_3 mediate their effects by modulating the expression of specific genes through binding to specific nuclear receptors which are transcription factors (RAR and TR, respectively). Moreover, signaling pathways of retinoids and thyroid hormones interplay and recent studies indicate that heterodimeric nuclear receptors utilize several mechanisms for increasing the complexity of transcriptional response [6].

Several years ago, RA [7] and T_3 [review in [8]] were established to be indispensable for the development of the central nervous system of vertebrates. Then, it was demonstrated that TR is expressed in numerous regions of the adult rat brain [9]. More recently it has been shown that the adult brain synthetizes RA, suggesting that retinoids may play an important role in the adult central nervous system [10]. The brain exhibits a selective distribution of cellular retinoid-binding proteins [11] and of nuclear receptors [12]. Among the RAR, RARβ is the main isoform expressed in the mature brain and this receptor is up-regulated by RA. It has been suggested that RARβ is involved in physiological functions and that these functions are regulated by RA concentration in the adult brain [12].

Thus, the roles of RA and T_3 in the developing, as well as the adult brain, are now established but there is little data concerning the involvement of these hormones in the aged brain. The aim of this investigation, therefore, was to study the influence of aging on the expression of RAR and TR in rat brain. The abundance of RAR and TR mRNA was measured and the activity of tissue transglutaminase (tTG) was assayed because the gene coding for this enzyme is regulated by retinoids, so that the enzymatic activity is considered to be an indicator of retinoid action [13,14]. Finally, the effect of administration of RA or retinyl palmitate (RP) (24 h before killing) in rats (6 or 24 months old) on RAR and TR messengers and enzymatic activity was studied.

2. Materials and methods

2.1. Experimental design

Official French regulations for the care and use of laboratory animals were followed. Weanling male Wistar rats were obtained from IFFA CREDO (L'Arbresle, France). Rats were housed four to a cage, in an air-conditioned room with a mean temperature of 21°C and a photoperiod which followed the seasonal pattern. Rats were randomly divided into seven groups designated as Young, Adult, Aged, Adult+RP, Adult+RA, Aged+RP and Aged+RA. Young rats were studied at 10 weeks of age, adult rats at 6 months and aged rats at 24 months. Adult+RA and Adult+RP were adult rats treated with 5 mg of retinoic acid (all-*trans* RA; Sigma no. R 2625)/kg body weight or 13 mg of retinyl palmitate (Sigma no. R 3375)/kg body weight, respectively, using an intragastric intubation 24 h before being killed. Aged+RP and Aged+RA were aged rats treated as Adult+RP and Adult+RA, respectively. Rats were fed a semi-synthetic diet prepared by the 'Atelier de préparation d'aliments expérimentaux' (Institut National de la Recherche Agronomique INRA, 78350 Jouy en Josas, France) according to published recommendations on the feeding conditions of laboratory animals [15] and contained 8000 UI vitamin A/kg. Food and water were freely available. Body weights were recorded throughout the experiment. Rats were killed by decapitation (between 09:00 and 10:00 h) and the brains were rapidly removed and stored at −80°C for subsequent analysis (mRNA quantification and cytosol preparation).

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PII S0014-5793(97)00845-4

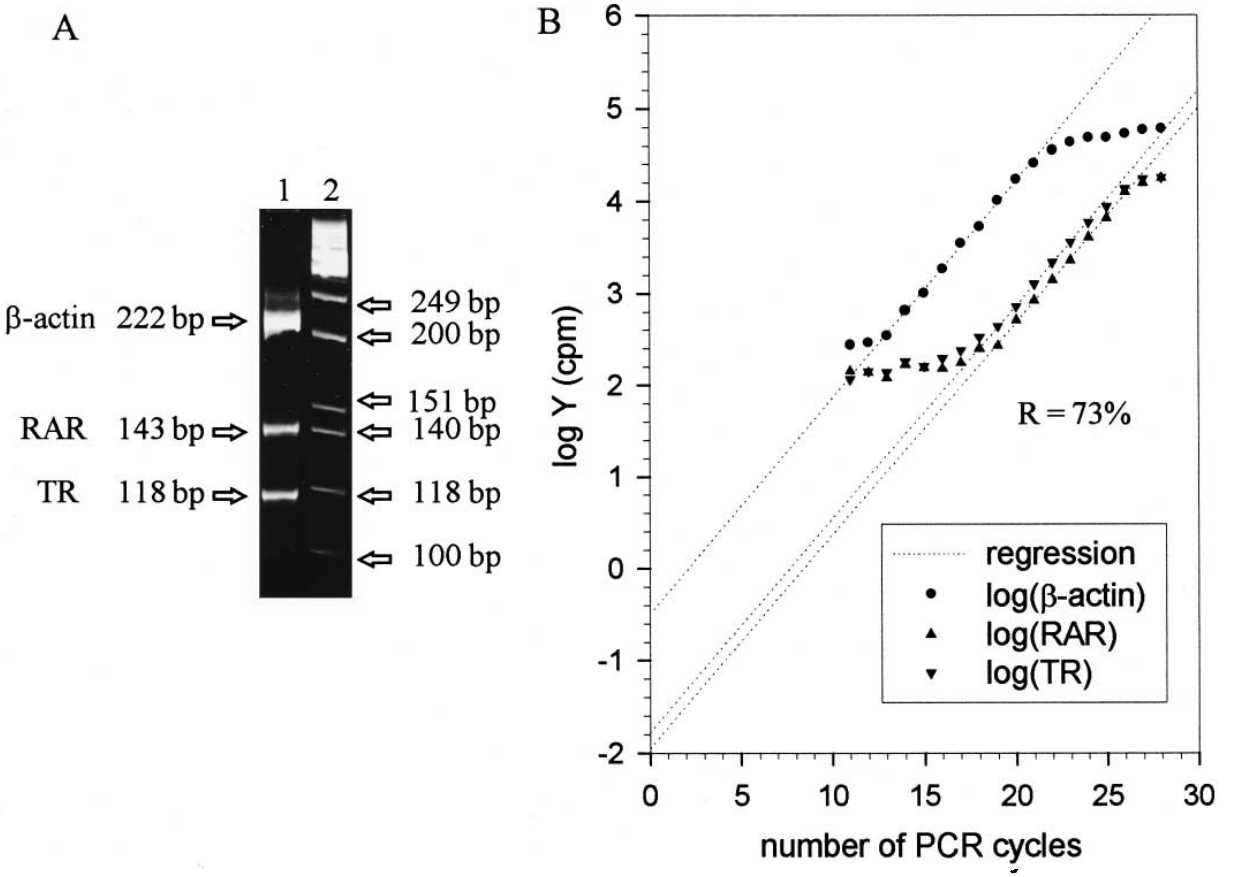

Fig. 1. Relative quantification of RAR and TR transcripts in rat brain. A: RT-PCR products resolved on the 10% acrylamide gel electrophoresis and stained with ethidium bromide. Lane 1, co-amplification of β-actin, RAR and TR transcripts in rat brain. Lane 2, molecular size markers ϕX174/hinfI. B: Semi-logarithmic representation of the relative extent of amplification (Y) measured by counting the amount of [α^{32}P]dCTP incorporated; R is the PCR efficiency.

2.2. Quantification of mRNA

mRNA was quantified by reverse transcription and amplification by the polymerase chain reaction (RT-PCR). The values of RAR and TR mRNA were obtained by comparison with levels of an internal standard, β-actin, that was simultaneously reverse-transcribed and amplified in the same test tube. The constancy of the level of β-actin mRNA with aging [16] was verified in our experimental conditions using the PCR MIMIC™ Construction Kit (Clontech Laboratories, Palo Alto, CA). Moreover, it is known that RA administration does not affect β-actin mRNA [17]. The conditions of extraction of mRNA, preparation of cDNA, amplification and quantitative analysis of PCR products have been described elsewhere [18]. The determination of the proportion of RAR and TR mRNA to β-actin mRNA was calculated according to Chelly and al. [19]. The oligonucleotide primers for RARβ, TRα1 and β1 (messengers encoding for proteins which bind T_3) are described in Table 1. Primers were purchased from GENSET (Paris, France). Fig. 1 shows, as an example, the results of an electrophoresis of PCR products of rat transcripts of *β-actin*, *TR* and *RAR* genes and also, a semi-logarithmic representation of the relative extent of amplification measured by counting the amount of ^{32}P incorporated.

2.3. Tissue transglutaminase assay

Brain homogenates (30%, w/v) for the tTG assay were prepared in 50 mM Tris-HCl buffer (pH 7.0) containing 0.25 M sucrose and 1 mM EDTA. After centrifugation at 105 000 × *g* for 60 min, the supernatant was collected for enzyme assay which was performed by detecting the incorporation of [^{3}H]putrescine into *N,N′*-dimethylcasein according to the method of Piacentini et al. [23].

Table 1
Sequences of oligonucleotide primers and size of amplified fragments

Primers	Sequences	Complementary sites	Size of amplified fragments(bp)
β-actin[a]	A1: AGGATGCAGAAGGAGATTACTGCC	2814–2837	222
	A2: GTAAAACGCAGCTCAGTAACAGTCC	3159–3135	
RAR[b]	R1: CTCACTGAGAAGATCCGGAAAGCCCACC	538– 565	143
	R2: TTGGTGGCCAGCTCACTGAATTTGTCCC	680– 653	
TR[c]	E1: TCCTGATGAAGGTGACGGACCTGC	1247–1270	118
	E2: TCAAAGACTTCCAAGAAGAGAGGC	1364–1341	

[a]From rat cytoplasmic *β-actin* gene according to the sequence of Nudel et al. [20].
[b]From murine RAR cDNA according to the sequence of Zelent et al. [21].
[c]From rat TR cDNA according to the sequence of Murray et al. [22].
Primers A1 and A2 were chosen in two different exons, the size of the PCR products provided a means to verify that the amplified fragment was not derived from genomic DNA.

Table 2
Influence of aging on the abundance of retinoic acid (RAR) and triiodothyronine (TR) nuclear receptor mRNA and on tissue transglutaminase activity (tTG) in whole rat brain

	2.5 months	6 months	24 months
RAR mRNA ($A_{RAR}/A_{\beta-actin}$ %)[a]	3.5± 0.1	3.4± 0.1	2.6± 0.1[c,d]
TR mRNA ($A_{TR}/A_{\beta-actin}$ %)[a]	4.9± 0.2	3.3± 0.2[c]	2.6± 0.2 [c,d]
tTG activity (fmol/h/mg prot.)[b]	180 ±10	188 ±20	118 ±10[c,d]

[a]Data represent the mean values ± SEM from 4 different pools of 2 rats.
[b]Data represent the mean values ± SEM from 8 rats.
Significantly different from ([c]) 2.5- or ([d]) 6-month-old rats (ANOVA followed by Tukey's multiple range post hoc test).

3. Results

3.1. Effect of aging on TR and RAR abundance

Table 2 shows that in the brain of aged rats (24 months old) the amounts of RAR and TR mRNA were lower (−26 and −47%, respectively) than in the brain of young rats (2.5 months). Moreover, the tTG activity was reduced in the brain of aged rats by approximately 34% relative to young rats. In the adult rats (6 months old), a lower amount (−32%) of TR mRNA was observed.

3.2. Effect of retinyl palmitate or retinoic acid administration

3.2.1. Adult (6 months old) rats. Twenty-four hours after RP administration, the abundance of TR and RAR mRNA and the tTG activity were not affected. On the other hand, after RA administration the amount of TR mRNA increased by about 20% and that of RAR mRNA by about 30% (Table 3).

3.2.2. Aged (24 months old) rats. RP administration had no effect on the abundance of TR mRNA while an increased RAR mRNA content (+30%) was observed. Following RA administration increased amounts of RAR and TR mRNAs (approximately +40%) and an increased tTG activity (+115%) were observed.

4. Discussion

The lower amount of TR mRNA in the brain of aged rats is in agreement with the results of De Nayer et al. [24] showing a decreased maximum binding capacity of TR in the brain of aged rats. The present data demonstrate, moreover, that this decreased expression of TR in aged animals resulted, at least in part, from a transcriptional event. This finding could be related to results obtained in Alzheimer cases (disease regarded as a pathological form of aging) showing a decreased level of TR mRNA, particularly of the sub-type TRα, in Alzheimer hippocampus [25]. Data on the influence of T_3 in the regulation of expression of *TR* genes are few but it has been shown that *TR* genes have a positive responsiveness to T_3 administration [26], and that thyroid hormone up-regulates *TRβ* gene expression in rat cerebral hemisphere astrocyte cultures [27]. Such data suggest that the decreased expression of the *TR* gene in aged brain may result from a reduction of the bioavailability of T_3 at the nuclear level.

The lower abundance of RARβ mRNA observed in the brain of aged rats may be considered to be the result of either a decreased RA bioavailability (which becomes insufficient to ensure the up-regulation of RARβ by RA), or an inability of RA to up-regulate its own receptors in a deficient T_3 status. It is known that, when T_3 is deficient, TR without ligand (apo-receptors) blocks the activity of RAR [28]. A decreased expression of RAR in the liver of hypothyroid rats has previously been reported [26,29].

The decreased activity of tTG, an enzyme the gene of which is a target of retinoids, is in agreement with the decreased abundance of RARβ mRNA. The lower expression of tTG in old rats may have neurobiologic consequences. Indeed, it is known that this Ca^{2+}-dependent enzyme induces covalent cross-linking of neuronal, probably dendritic, proteins and that such a binding mechanism may be part of the long-term potentiation process (LTP) [30]. The LTP synaptic transmission is used as an experimental model for investigating the synaptic basis of learning and memory [review in [30]].

In a second part of this study, acute treatment with RA or RP was used to investigate restoration of youthful levels of mRNA of these nuclear receptors in aged rats. In aged rats, 24 h after RA administration, increased abundances of RAR as well as TR mRNA were observed. These results demonstrate that the expression of *RAR* and *TR* genes, in the brain, which decreases with age, can be up-regulated by RA as in

Table 3
Effect of retinyl palmitate (RP) or retinoic acid (RA) administration on RAR and TR mRNA abundance and on tissue transglutaminase (tTG) activity in the brain of 6- (adult) or 24-month-old (aged) rats

	Adult rats			Aged rats		
	Basal	+RP	+RA	Basal	+RP	+RA
RAR mRNA ($A_{RAR}/A_{\beta-actin}$ %)[a]	3.4± 0.1	3.4± 0.2	4.4± 0.4*	2.7± 0.1	3.5± 0.3*	3.8± 0.1*
TR mRNA ($A_{TR}/A_{\beta-actin}$ %)[a]	3.3± 0.2	3.0± 0.2	4.0± 0.2*	2.6± 0.2	2.9± 0.2	3.7± 0.1*
tTG activity (fmol/h/mg/prot.)[b]	188 ±16	152 ±20	214 ±16	118 ±12	160 ±14	254 ±12*

[a]Data represent the mean values ± SEM from 4 different pools of 2 rats.
[b]Data represent the mean values ± SEM from 8 rats.
*Significantly different from non-treated old rats (ANOVA followed by Tukey's multiple range post hoc test).

adult rats. Moreover, tTG activity, which is a good indicator of RA action [13,14], and which is low in aged rats, is also induced by RA. The observation of an up-regulation of TR mRNA by RA in the brain of aged rats constitutes an additional example of interaction between the signaling pathways of RA and T_3. The influence of vitamin A status on TR expression in the liver has previously been shown [31]. The mechanism responsible for this kind of heteroregulation of TR transcription has not been established but RXR (nuclear receptor the ligand of which is the 9-*cis* RA isomer) may be involved because TR and RAR form heterodimers with the common partner RXR [32].

The dose of RP used had no apparent effect on adult rats but was able to induce an up-regulation of RAR in aged rats (whose RAR mRNA abundance is lower). Thus, in the conditions of this study, the responsiveness of RAR to RP administration would seem to depend on the level of receptor expression. RA induced an increase in RAR mRNA abundance in aged rats but also in adult rats because RA is a more potent retinoid than RP.

In summary, we showed that *RAR* and *TR* genes, which are involved during the development of the brain, are under-expressed in the older brain but this level of expression can be restored by retinoid administration. New studies are required to identify the molecular targets of RA and T_3 and particularly the genes for which RAR and TR are transcription factors. The present study confirmed that tTG is modulated by RA but there are numerous putative proteins which could also be neuronal targets for RA and T_3 in the aged brain. Among these proteins, it will be interesting to study a neuronal specific protein, RC3 (neurogranin), because this protein is under the influence of thyroid hormone in vivo [33] and because a RA response element (RARE) has been described in its gene [34]. RC3 is related to dendritic spine formation and synaptic plasticity so that it could be hypothesized that in the aged brain a deficiency of T_3 and/or RA expression is responsible for a low level of RC3 and subsequently for a deficient synaptic plasticity.

Acknowledgements: This study was supported in part by an 'Aide Individuelle Programmée' of the 'Institut National de la Recherche Agronomique' and the 'Pôle Médicament Aquitaine'.

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