The Early Decrease in N-Acetyl Aspartate / Glutathione, a Brain Health Marker, Induced by Vitamin A Deprivation in Rat is Reversed by Retinoic Acid†

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Abstract: Retinoids are involved in adult brain function and in neurodegenerative diseases. Decreased expression of synaptic plasticity markers and metabolic changes induced by 14 weeks of vitamin A deprivation (VAD_{14}) were previously evidenced in rat brain. We evaluate early metabolic changes (by HRMAS proton NMR spectroscopy) on biopsies of cortex, hippocampus and striatum (i) during VAD and (ii) after *all trans* Retinoic Acid administration.

In 10 wk VAD (VAD_{10}), metabolic changes appeared in the cortex: (i) N-acetyl aspartate (NAA, +15%, P=0.05 *vs* C_{10}), a neuronal density indicator, (ii) glutathione (GSH, +30%, P=0.05 *vs* C_{10}), an antioxidant status marker. Concerning the impact of VAD duration, the NAA/GSH ratio in cortex, hippocampus and striatum was unchanged in all controls, whereas it decreased in striatum and hippocampus of VAD_{10} and in striatum and cortex of VAD_{14}. ATRA had an apparent regulatory action by increasing NAA/GSH ratio in hippocampus ($VAD_{10+ATRA}$ *vs* VAD_{10}: +34%, P=0.03), the striatal ratio being rescued to control level. Gene expression of BACE and APP, which are amyloidogenesis markers, were unchanged in VAD_{10} whereas VAD_{14} seemed to activate cortical amyloidogenesis.

Hypoexpression of retinoid signaling during a short 10 wk period has consequences on brain metabolic profile and precedes the impaired expression of both amyloidogenesis and synaptic plasticity markers. Rat nutritional VAD represents a model of accelerated aging which mimics the course of aging. The NAA/GSH ratio reflects the balance between neuronal health and protection against reactive oxygen species, and could thus serve as a marker of brain health.

Keywords: HRMAS, vitamin A deprivation, retinoic acid, brain metabolism, antioxidant status, neuronal health.

INTRODUCTION

Retinoids are a family of compounds derived from vitamin A that have several important functions in many tissues including a role in vision, maintenance of epithelial surfaces, immune competence, reproduction, and embryonic growth and development [1]. The majority of these functions (excluding the extravisual functions) are controlled by the vitamin A metabolite retinoic acid (RA), which binds to receptors of the nuclear receptor superfamily and regulates gene expression [2]. It is well known that retinoids, and particularly RA, play an important role during the development of the Central Nervous System (CNS) (as review by [3]). However, to date, the role of retinoids in the adult CNS is less understood and has only recently attracted scientific attention. Some data suggest that fine regulation of retinoid-mediated gene expression seems fundamentally important for optimal brain functioning such as long-term potentiation (LTP), which is strongly affected by changes in vitamin A availability [4]. Previous studies have shown its role in synaptic plasticity, learning and memory [4-9]. Recently, data from a number of studies have suggested the involvement of retinoid signaling in the etiology of neurodegenerative disorders like Alzheimer or Parkinson diseases [10-13].

The adult brain possesses all the machinery to metabolize and produce RA from retinol supplied by the blood. The Retinoic Acid Receptor (RAR) is a DNA-binding protein which, upon activation by specific RA ligands, induces gene transcription by interacting with distinct promoter sequences in the target genes. Among the many genes whose expression is regulated by RA, there are those coding for two identified neurone-specific kinase susbtrates involved in synaptic plasticity, i.e. neurogranin or RC3 and neuromodulin or GAP 43. In the brain, the highest levels of RA were observed in the striatal region, which strongly expressed (RARβ), one of the RAR isoforms. The hippocampus, a region highly involved in neuronal processes such as synaptic plasticity, long-term depression (LTD) and LTP, is strongly affected by changes in vitamin A availability [4]. Previous studies have

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shown the presence of retinoid-specific receptors in the hippocampus and have demonstrated that vitamin A deficiency produces a severe deficit in spatial learning and memory, which are linked to proper hippocampal hypofunctioning [8]. In aged mice where RA signaling is hypoactivated in the brain, supplemental RA can help to reverse the age-related decrease in hippocampal LTP [9]. On the other hand, in aged rats, the retinoid signaling pathway was also hypoactivated and found to be associated with memory disorders [6, 14]. Many of our laboratory studies have already shown the beneficial effect of RA administration on neuronal functions. The ability of RA to rescue spatial learning and memory deficits in aged animals or in the transgenic Alzheimer model [8, 9, 15] suggests that activation of retinoid signaling constitutes a potential therapeutic strategy to alleviate adult brain functioning and memory. In the transgenic mouse model of AD, RA treatment reduced amyloid β accumulation and Tau protein hyperphosphorylation, two specific marks of the disease [15]. Moreover, contents of RARβ and proteins involved in the amyloidogenic pathway, amyloid precursor protein (APP695) and β-secretase enzyme (BACE), have been observed to decrease in the whole brain and in the cerebral cortex in 13 wk VAD rats. Administration of RA is able to restore all expression, suggesting that fine regulation of vitamin A-mediated gene expression seems fundamental for the regulation of APP processing [13].

It is known that in major biological processes, gene expression disturbances are associated with metabolic changes. Few metabolic studies have been performed using High Resolution Magic Angle Spinning (HRMAS). Proton Nuclear Magnetic Resonance (NMR) spectroscopy is a technique which offers the simultaneous high resolution on small brain biopsies with highly resolved spectra [16, 17]. Recently, in rats after 14 wk VAD from weaning, we evidenced brain metabolic changes known to be associated with neurodegenerative disorders [18]. Expression of genes linked to synaptic plasticity was also strongly affected after 14 wk VAD [19]. We also showed in longitudinal Magnetic Resonance Imaging (MRI) studies that VAD induced early (between 7 and 9 wk) major anatomical disturbances in rat brain. These changes were highly correlated with retinol status [18].

Our main purpose was to evaluate the effect of a shorter VAD (10 wk) on the brain metabolic profile using ^{1}H HRMAS spectroscopy. Since it has already been shown that biological processes can be reversed at a moderate stage of VAD [13], we also examined the effect of *all-trans* RA short treatment on the neurochemical profile of cortex, hippocampus and striatum of 10 wk VAD rats.

MATERIALS AND METHODOLOGY

Animal Experiments

Weaned male Wistar rats (initial weight 45-50g) were purchased from Harlan (France). They were housed in individual hanging cages in a room with constant airflow system, controlled temperature (21-23°C) and hygrometry and a 12 hr light/dark system. They were divided into four experimental groups: vitamin A-deficient group for 10 weeks (VAD_{10}) (n=10), vitamin A-deficient group for 14 weeks (VAD_{14}) (n=5), control group (n=10) for 10 weeks (C_{10}) and control group for 14 weeks (C_{14}) (n=5). The control diet was the same as the VAD diet (Harlan, Gannat, France) plus vitamin A (1515 RE/kg diet or 1.15µg retinol/g diet) (Table **1**).

Table 1. Formulation of Free Vitamin A Rat Diet[1] (Energy Value 15.9KJ/g)

Ingredient	Amount g/kg
Vitamin-free casein	193
Sucrose	510
Corn Starch	150
Cottonseed oil	50
Cellulose	50
Mineral mix[2]	43.49
Vitamin mix and Choline[3]	5.05
Protein	177
Carbohydrate	649
Fat	50

[1]VAD diet from Harlan (France). Diets were stored in scaled bags at 4°C.
[2]Composition of mineral mix (g/kg): mineral mix AIN-76 (170915), 35; calcium carbonate $CaCO_3$, 4; Choline dihydrogene citrate, 3.497.
[3]Composition of vitamin mix (g/kg): DL-α-tocopherol acetate, 0.121; dry vitamin D3 (500000U/g), 0.0044; ascorbic acid, 1.0166; biotin, 0.0004; vitamin B12, 0.0297; calcium panthothenate , 0.0661; folic acid, 0.002; inositol, 0.1101; menadione, 0.0496; niacin, 0.0991; pyridoxine HCl, 0.022; riboflavin, 0.022; thiamin HCl, 0.022.

At the 10th wk of diet, in order to study the effect of RA at an early VAD stage, half of the VAD_{10} and C_{10} groups, named respectively $VAD_{10+ATRA}$ and $C_{10+ATRA,}$ were injected daily for four days with *all-trans* RA (ATRA, 150 µg of *all-trans*-RA/kg, Sigma, France) dissolved in a vehicle containing polyethylene glycol-NaCl-ethanol (70:20:10, vol:vol). This dose of ATRA was shown to be effective in reversing age-related hypoexpression of retinoid brain signaling and its associated memory impairment [9] in mice and in restoring expression of genes associated with synaptic plasticity (as neuromodulin and neurogranin) directly regulated by RA in VAD rats [8, 19]. The other half of the groups ($C_{10,}$ VAD_{10}) was treated daily with vehicle alone for four days. Animals had *ad libitum* access to food and tap water. All animals were weighed every two days to monitor body weight.

The study complied with 1999 UFAW guidelines [20]. The protocol for these experiments was approved by the Ethics Committee for Animal Experiments in our university.

HRMAS ^{1}H NMR Spectroscopy

Rats from all groups were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 ml/kg of body weight). A longitudinal incision was rapidly made on the scalp of the animals to reveal the cranium. Brains were then frozen *in situ* by the "funnel freezing" method as described by Pontén *et al.* [21], in order to avoid post-mortem failure of energy metabolism. After instant decapitation with a scientific guillotine, brain was then removed and kept at -80°C. Proton HRMAS NMR spectroscopy using a DPX 500

Brucker (50 µL rotor) operating at 11.7 T was performed on samples (20-30 mg) of cortex, hippocampus and striatum maintained at 0°C throughout the experiment. Each sample was placed in a 50 µl tube (Brucker Biospin Ltd, Germany), 20 µL of D_2O was added. The total relaxed spectrum with suppression of water resonance was acquired (size 32 K, number of scans=64, total acquisition time=12min 37s, relaxation time 8 s). The content of metabolites such as N-acetyl aspartate (NAA, 2.01 ppm), total glutathione (GSH, 3.77 ppm), choline (Cho, 3.18 ppm), taurine (Tau, 3.25 ppm) and glutamate-glutamine (2.05 to 2.15 ppm) was analyzed with Brucker software XWIN NMR to obtain relative contents to total creatine (Cr, 3.01 ppm; including Creatine + Phosphocreatine).

Measurement of Serum Retinol Concentration

After sacrifice, blood was collected (400 µL) and spun at 3000 rpm for 15 min. The supernatant was removed and snap-frozen on dry ice. Serum retinol was assayed by high performance liquid chromatography according to a previously described method [22].

mRNA Expression of APP695 and BACE

As already reported [22], the mRNA expression of some amyloidogenesis markers is not affected in VAD_{10}. Hence some markers of amyloidogenesis such as mRNA of APP695 and BACE were measured in cortex and hippocampus of VAD_{14} only. mRNA levels were obtained from reverse transcription and polymerase chain reaction (RT-PCR, using PPIB: peptidylprolyl isomerase B (cyclophilin B) as a house-keeping gene), as previously described [19].

Statistical Analysis

All results were expressed as mean±SEM. Data were submitted to analyses of variance. When appropriate, post-hoc comparisons were performed using the Tukey test. Whenever two groups were compared, an unpaired t-test was used.

RESULTS

Effect of Vitamin A Deprived Diet on Body Weight

For the groups VAD_{10} and $VAD_{10+ATRA,}$ rats were deprived of vitamin A for 10 wk from weaning since it had previously been demonstrated that such a diet duration was sufficient to evidence the effects of VAD [19, 23]. A curtailment of growth was observed in the VAD groups from wk 7-8 and became significantly different from wk 9 ($P=0.0025$) *vs* C_{10} (Fig. **1**). No alteration of general health was observed. No effect of ATRA injections on body weight was observed in $C_{10+ATRA}$ and in the $VAD_{10+ATRA}$. A loss of body weight was observed at the end of deprivation in the VAD_{14} group while body weight continuously increased in the C_{14} group (223±11g and 381±8.5g, respectively), as previously described [18].

Status of Vitamin A Deficiency

At sacrifice, serum retinol concentration was significantly lower in VAD_{10} (0.12±0.09 µmol/l) *vs* C_{10} (2.12±0.1 µmol/l, $P<0.0001$) rats. In VAD_{14} rats, serum retinol concentration was undetectable [18], while retinol level in C_{14} animals was unchanged compared to C_{10} rats.

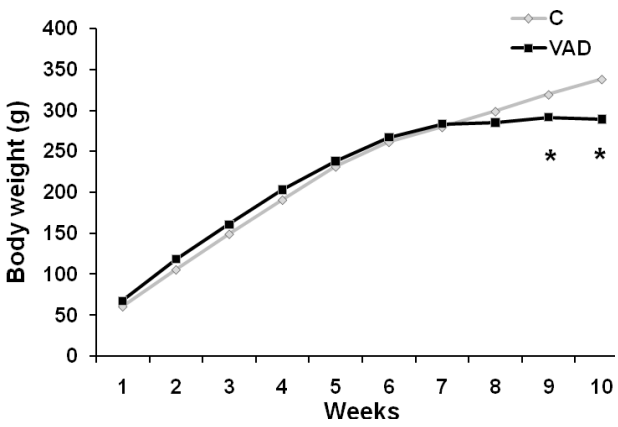

Fig. (1). Changes in body weight in vitamin A-deprived rats and control rats during 10 wk after weaning. For each point, n=10, means±SEM. ANOVA with post-hoc Tukey test. For each time, diet groups marked with an asterisk are significantly different ($P<0.05$).

Relative Contents of Brain Metabolites

Relative content of metabolites was evaluated from NMR spectra. Two typical ^{1}H HRMAS NMR spectra from brain striatum in C_{10} rats and in VAD_{10} rats are shown in Fig. (**2A** and **2B**), respectively.

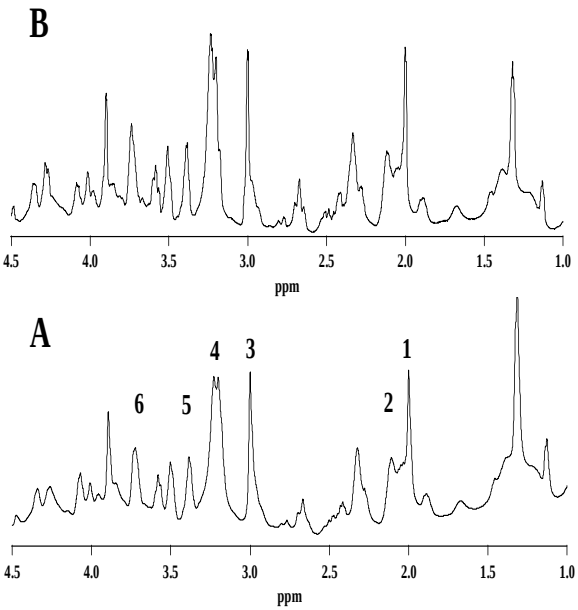

Fig. (2). Typical ^{1}H HRMAS NMR spectra from brain striatum in control (A) and VAD (B) rats at 10 weeks of experiment. NMR spectra peak assignments: 1: N-acetyl aspartate (NAA), 2: glutamate+glutamine (Glx), 3: creatine+phosphocreatine (Cr), 4: choline (Cho), 5: taurine (Tau), 6: glutathione (GSH).

Effect of 10 Weeks of VAD on Neurochemical Profile

Relative metabolite contents after 10 wk of experiment are shown in Table **2**.

Table 2. Effect of ATRA on Relative Content of Brain Metabolites in Rats at the End of 10 Weeks of Experiment

Metabolites	VAD_{10}	$VAD_{10+ATRA}$	C_{10}	$C_{10+ATRA}$
NAA/Cr				
CX	**1.16 ± 0.059***	**1.01 ± 0.019#**	1.01 ± 0.051	1.01 ± 0.061
H	0.92 ± 0.035	**1.06 ± 0.072#**	0.93 ± 0.042	1.00 ± 0.055
S	0.85 ± 0.078	0.98 ± 0.080	0.88± 0.080	1.02 ± 0.073
GSH/Cr				
CX	**0.39 ± 0.038***	0.38 ± 0.027	0.30 ± 0.028	0.29 ± 0.023
H	0.36 ± 0.037	**0.29 ± 0.017#**	0.32 ± 0.046	0.33 ± 0.026
S	0.38 ± 0.026	0.38 ± 0.050	0.32 ± 0.032	0.38 ± 0.033
Cho/Cr				
CX	1.03 ± 0.194	0.93 ± 0.132	0.68 ± 0.114	0.98 ± 0.118
H	1.08 ± 0.148	0.93 ± 0.083	0.79 ± 0.125	0.95 ± 0.125
S	0.87 ± 0.139	0.86 ± 0.128	0.83 ± 0.090	1.08 ± 0.125
Tau/Cr				
CX	0.36 ± 0.029	0.30 ± 0.024	0.37 ± 0.071	0.34 ± 0.027
H	0.29 ± 0.030	0.23 ± 0.018	0.28 ± 0.021	0.27 ± 0.039
S	0.41 ± 0.020	0.32 ± 0.047	0.36 ± 0.031	0.41 ± 0.055
Glx/Cr				
CX	**0.44 ± 0.061***	0.35 ± 0.033	0.33 ± 0.108	0.40 ± 0.070
H	0.34± 0.059	0.33 ± 0.046	0.37 ± 0.033	0.43 ± 0.053
S	0.39 ± 0.053	0.39 ± 0.064	0.36 ± 0.039	0.44 ± 0.025

Expression as ratio to creatine+phosphocreatine (Cr) in control rats (C_{10}) and vitamin A-deprived rats (VAD_{10}): N-acetyl aspartate (NAA), glutathione (GSH), choline (Cho), taurine (Tau), gamma amino butyric acid (GABA) and glutamate-glutamine (Glx) in cortex (CX), hippocampus (H) and striatum (S). Means±SEM (n=5 for each group). ANOVA followed by a post-hoc Tukey test. For each brain structure, diet groups significantly different for the metabolite considered were marked with * (between VAD and C) and with # for ATRA effect (P<0.05).

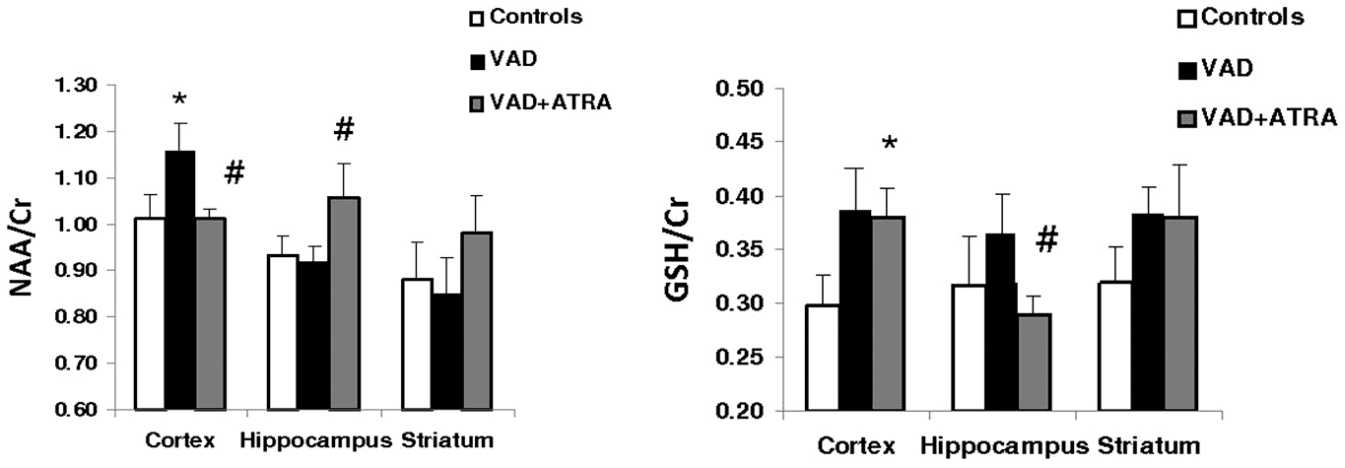

Fig. (3). Effect of ATRA on relative content of N-acetyl aspartate (NAA) and glutathione (GSH) in VAD and control rats. Measurement at 10 wk of experiment expressed as ratio to creatine+phosphocreatine (Cr). Means±SEM (n=5 for each group). ANOVA followed by a post-hoc Tukey test. For each brain structure * describes significant difference between VAD and control groups and # significant difference between VAD and VAD+ATRA group (P<0.05).

Metabolic changes observed after 10 wk of VAD mainly concerned the cortex with an increase in (i) NAA (+15%, P=0.05 *vs* C_{10}), the indicator of neuronal density (Fig. **3**), (ii) GSH (+30%, P=0.05 *vs* C_{10}), the marker of antioxidant status (Fig. **3**), and (iii) Glx (+33%, P=0.05 *vs* C_{10}), a neurotransmitter and brain energy substrate (Table **2**).

To investigate the balance between neuronal health and protection against reactive oxygen species (ROS), the NAA/GSH ratio was calculated. In cortex, hippocampus and striatum, it remained similar in C_{10} and C_{14} rats. VAD induced a greater or lesser decrease in the NAA/GSH ratio in the three brain structures. The phenomenon reached significance in the striatum and hippocampus of VAD_{10} rats (Fig. **4**).

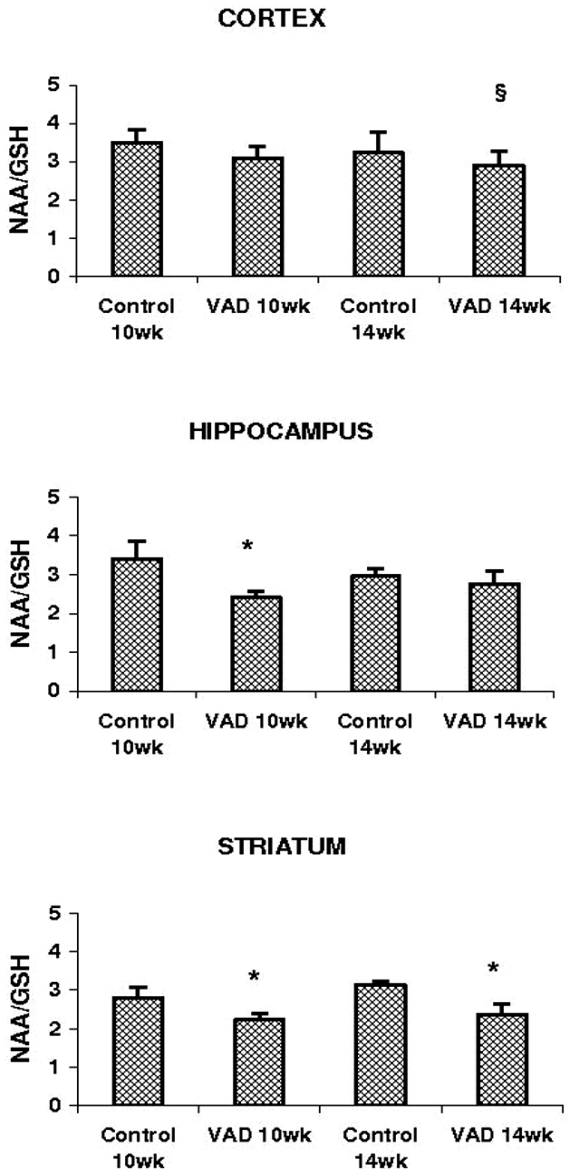

Fig. (4). N-acetyl aspartate (NAA) / glutathione (GSH) ratio. Determination in cortex, hippocampus and striatum of control rats and VAD rats after 10 wk or 14 wk of experiment. Means±SEM (n=5 for each group). ANOVA followed by a post-hoc Tukey test. P<0.05: * VAD *vs* Control for the same experimental time, and § VAD_{14} *vs* C_{10}.

Effect of Retinoic Acid on Neurochemical Profile in 10-Week Experiment

In control rats, ATRA induced no significant change (Table **2**) so ATRA had no effect on the NAA/GSH ratio in the three cerebral structures (Fig. **5**).

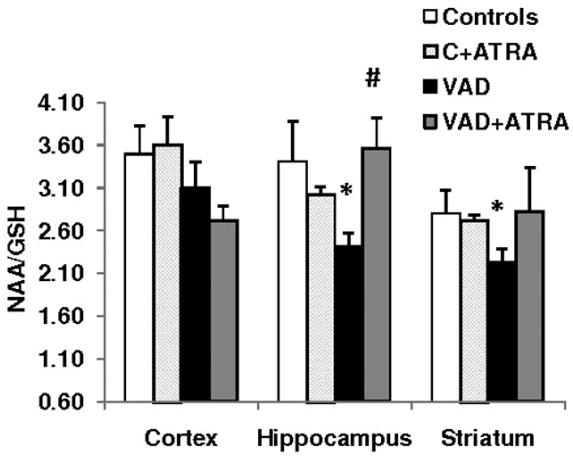

Fig. (5). Effect of retinoic acid (ATRA) on N-acetyl aspartate (NAA) / glutathione (GSH) ratio. Determination in cortex, hippocampus and striatum of control rats and VAD rats after 10 wk of experiment. Means±SEM (n=5 for each group). ANOVA followed by a post-hoc Tukey test. P<0.05: # $VAD_{10+ATRA}$ *vs* VAD_{10}, * VAD_{10} *vs* C_{10}.

In VAD_{10} rats, ATRA decreased the cortical NAA/Cr ratio (-12%, P=0.025 *vs* VAD_{10}) (Table **2**) which was rescued in C_{10} rats (Fig. **3**). Moreover, an increase in NAA/Cr (+17%, P=0.05 *vs* VAD_{10}) and a decrease in the GSH/Cr ratio (-19%, P=0.05 *vs* VAD_{10}) was observed in hippocampus (Fig. **3**). No significant effect of ATRA was observed on the GSH/Cr ratio (Fig. **3**) in the two other brain structures (Table **2**). The NAA/GSH ratio was significantly increased in hippocampus ($VAD_{10+ATRA}$ *vs* VAD_{10}: +34%, P=0.03) and was rescued to the same level as in C_{10} rats in striatum (Fig. **5**).

Markers of Amyloidogenesis

The mRNA expression of some markers of amyloidogenesis was affected in VAD_{14} rats. There was a significant decrease in APP695 in the cortex and a significant increase in BACE in the cortex and hippocampus (Table **3**).

Table 3. Effect of 14 wk Vitamin A-Deficiency on mRNA Expression of APP695 and BACE in the Hippocampus and Cortex of Rat Brain

	APP 695 mRNA (% PPIB)	BACE mRNA (% PPIB)
Cortex		
Controls	100 ± 7	100 ± 6
VAD_{14}	63 ± 5*	130 ± 7.2*
Hippocampus		
Controls	100 ± 7.3	100 ± 5.6
VAD_{14}	85 ± 10	140 ± 4.3*

m±SEM, n=7. * P<0.05 *vs* control rats.

DISCUSSION AND CONCLUSION

The main purpose of the present work was to evaluate early changes in the neurochemical profile of the cortex, hippocampus and striatum in vitamin A-deprived rats and the effect of *all-trans* RA treatment in 10 wk VAD rats.

Classically, one of the limitations for absolute quantification of metabolites is the choice of the reference. From a ^{1}H NMR spectrum, metabolites are classically and relatively quantified to the creatine+phosphocreatine peak [24, 25] assigned at a chemical shift of 3.01 ppm. Hence, the present work was conducted by monitoring relative changes in metabolite content. It is thus possible to draw valid conclusions because the ratio is not based on absolute quantification [18].

Many reports suggest the major functional involvement of retinoid signaling in synaptic plasticity, learning and memory, sleep, schizophrenia, depression, Parkinson disease, and Alzheimer disease (AD) [3, 11, 26, 27]. In a recent work, we showed that brain atrophy and metabolic changes during 14 wk of VAD administration in rats were highly correlated with retinol status [18]. Moreover, spatial memory deficits in the water maze test observed in 14 wk VAD rats could be reversed by RA administration [14]. The present data and especially those concerning the markers of neuronal health (NAA) and antioxidant status (GSH) demonstrate (i) early changes in the content of some metabolites (10 wks VAD) and (ii) that ATRA treatment is able to reverse these changes. Whatever their duration, the impairments in the NAA/GSH ratio induced by VAD are significant in the striatum, since it is the brain structure possessing all the necessary components for the synthesis and catabolism of retinoids.

Concerning the effect of VAD duration, the hypoexpression of RA receptors affected the mRNA expression of some amyloidogenesis markers by decreasing APP695 and increasing BACE expression only in the cortex, and only in VAD_{14} rats. This finding suggests that the prolongation of deprivation induces a progressive shift in the APP degradation pathway from a physiological process to a pathological one. Moreover, we have previously shown that 14 wk of VAD induced a significant decrease in the NAA/Cr ratio in cortex, hippocampus and striatum [18]. Here, unexpectedly, we evidenced that 10 wk VAD induced an increase in the NAA/Cr ratio only in cortex. The biological role of NAA is still unclear. Several functions may be hypothesized such as osmotic regulator, source of acetate for myelin synthesis or direct precursor of the neuromediator NAAG. In NMR, NAA is considered to be a marker of neuronal density, since it is mainly present in neurons but not in glial cells [28, 29]. It is now also viewed as a sensitive marker of neuronal viability and function, a decrease in the NAA/Cr ratio indicating neuronal suffering [30]. In most neurodegenerative disorders, the NAA/Cr ratio is diminished [31] except in Canavan disease which is a genetic childhood disease characterized by progressive cerebral demyelinization [32]. Recently, NAA synthesis was found to be higher in some AD patients [32, 33]. The authors hypothesized an adaptative process which could occur in the neurons of these patients. Here, we suggest that the same adaptative process could occur in rat cortex at 10 wk of VAD as an attempt to prevent the pathologic events that were characterized at 14 wk by a dramatic decrease in the NAA/Cr ratio.

GSH/Cr significantly increased in cortex after 10 wk VAD and tended to increase in hippocampus and striatum. Glutathione has many physiological functions including its involvement in the defense against reactive oxygen species (ROS). Glutathione cycles between the reduced form (GSH) and the oxidized disulfide form (GSSG), and serves as a vital molecule for controlling ROS levels in cells. With NMR it is impossible to distinguish the reduced form from the oxidized one. The reduced form reacts with oxygen- and nitrogen-free radicals and results in the reduction of peroxides [34-36]. GSH deficiency has been found in neurodegenerative brain diseases [37]. Although varying in different regions of the brain, all total glutathione levels diminish by about 30% in the elderly [38], suggesting a possible link with the age-associated risk factor of AD and Parkinson disease.

Strong links have been established between inflammation and AD, since inflammation might participate in AD by generating ROS [39]. Low-grade inflammation is also associated with age-related chronic diseases, and the accumulation of lysosome- and mitochondria-derived ROS in microglia are the most important causative factors of brain aging [40]. Interestingly, inflammation in rat leads to hyporetinolemia [41]. Links between retinoids and glutathione have already been made indirectly by Wu *et al.* [42] who showed that retinoic acid receptor RXRα regulates glutathione homeostasis by regulating expression of glutathione transferase and glutathione peroxidase. We suggest that the increase in the GSH/Cr ratio at 10 wk of VAD in the three structures may reflect an increase in ROS, which could be involved in a neuroinflammation process in this model of accelerated aging.

On the other hand, one of our findings was the decrease in the NAA/GSH ratio resulting from VAD. This probably reflects an imbalance between neuronal health and protection against ROS in the cortex, striatum and hippocampus, where strong retinoid signaling pathway activity has been reported in rodents [43, 44]. This imbalance is especially significant in the striatum, which is known to be the most responsive to vitamin A [19].

As with all vitamin deficiencies, we expected that reintroducing vitamin A *via* ATRA would reduce or reverse the changes observed in deprived rats. The results show that ATRA injections in 10 wk VAD rats had differential effects according to the brain structures. ATRA would seem to have a regulatory action by diminishing and rescuing metabolite content. It rescues NAA in the cortex and GSH in the hippocampus, the latter structure being particularly sensitive to retinoids [9, 14, 45]. On the other hand, ATRA regulated the NAA/GSH ratio in hippocampus and striatum, these structures being known to show decreased expression of the proteins involved in synaptic plasticity in ageing [46] and vitamin A deprivation [19].

It is known that the retinoic acid receptor RAR is a DNA-binding protein which, upon activation by specific RA ligands, induces gene transcription by interacting with distinct promoter sequences in the target genes. Among the many genes whose expression is regulated by RA, there are

those coding for two identified neuron-specific kinase substrates involved in synaptic plasticity, i.e. neurogranin or RC3 and neuromodulin or GAP 43. In 10 wk VAD rats, a decrease in RARβ expression was reported in the striatum [19] and was improved by ATRA treatment [13]. In complementary experiments in the same conditions, no modification was found in the cortex and hippocampus with regard to RC3 and GAP 43 expression [19], whereas this gene expression pathway was altered in VAD_{14} rats [47]. However, decreased amounts of GAP 43 and RC3 mRNA and proteins were found in the striatum of VAD_{10} rats, the reduction in the amounts of GAP 43 mRNA and protein being reversed by ATRA administration [19].

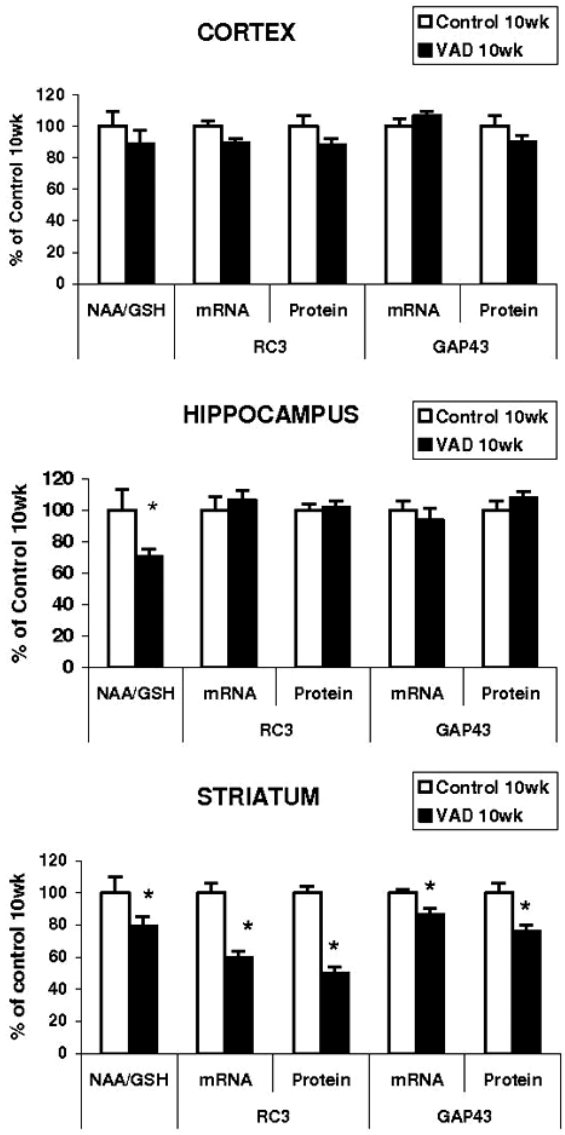

Fig. (6). Comparison of NAA/GSH and expression of RC3 (neurogranin) and GAP 43 (neuromodulin) (the latter from [47]). Measurements in cortex, hippocampus and striatum of VAD rats and control rats after 10 wk of protocol mRNA and protein levels were obtained from reverse transcription and polymerase chain reaction (RT-PCR (using GAPDH: glyceraldehyde-3-phosphate dehydrogenase as a house-keeping gene) and Western Blot, as previously described [19]. Data are expressed as % of expression of GAPDH for mRNA and as % of controls for proteins. Controls are expressed for all results as 100%. Means±SEM, *P<0.05 *vs* control group.

Comparison of the changes in both NAA/GSH ratio and gene expression underlines the differential response of brain structures to the action of retinoids (Fig. **6**). In striatum, the decrease in retinoid bioavailability induces similar changes in the NAA/GSH ratio and gene expression of RC3 and GAP 43, which can be reversed by ATRA. In cortex and hippocampus, VAD tends to control the NAA/GSH ratio whereas it has no effect on the signaling pathway of synaptic plasticity. The present findings show that this metabolic change can occur before the appearance of changes in gene expression, thereby highlighting the value of the NAA/GSH ratio as an early marker of brain activity disorders.

This study shows that deregulation of retinoid signaling by nutritional VAD even for a relatively short 10 wk period has consequences on the brain metabolic profile. If the treatment is extended to 14 wk, severe anatomic, metabolic and memory disorders occur [14, 18], which are accompanied by an imbalance in the amyloidogenic pathway but no increase in the amount of β amyloid peptides (personal data), amyloid deposits being observed only after one year of VAD [10].

Therefore, we suggest that VAD represents a rat model of accelerated aging that mimics what occurs during aging. The kinetic is as follows: Step 1 = 5-6 wk: curtailment of brain growth followed by a decrease in total brain volume, Step 2 = 10-11 wk: decrease in both hippocampal volume and NAA/GSH ratio, increase in ventricular spaces and onset of impairment in gene expression of synaptic plasticity, Step 3 = 13-14 wks: memory disorders, decrease in NAA/Cr ratio, changes in gene expression of amyloidogenesis but no deposits of amyloid peptides. This sequence of events has already been observed in an AD transgenic model where the decrease in hippocampal and cerebral volumes preceded amyloid deposits [48]. In our hypothesis, the NAA/GSH ratio is an early marker of the accelerated process of aging and reflects the balance between neuronal health and antioxidant status, which are systematically decreased in VAD_{10} and VAD_{14} rats.

In conclusion, vitamin A *via* its main active metabolite, RA, is involved in regulating brain metabolism and could therefore be considered as a key molecule for preventive strategies in neurodegenerative diseases [15].

ABBREVIATIONS

APP	=	amyloid precursor protein
ATRA	=	all trans Retinoic Acid
AD	=	Alzheimer disease
BACE	=	β secretase
C_{10} and C_{14}	=	controls rats at 10 and 14 wk of experiment
Cho	=	choline
CNS	=	central nervous system
Cr	=	creatine+phosphocreatine

GABA = gamma amino butyric acid

GAPDH = glyceraldehyde-3-phosphate dehydrogenase

GAP 43 = neuromodulin

Glx = glutamate-glutamine

GSH = glutathione

GSSG = oxidized glutathione disulfide

HRMAS = high resolution magic angle spinning

LTP = long-term potentiation

MRI = magnetic resonance imaging

NAA = N-acetyl aspartate

ND = neurodegenerative disorders

NMR = nuclear magnetic resonance

PPIB = peptidylprolyl isomerase B (cyclophilin B)

RA = retinoic acid

RAR = retinoic acid receptor

RC3 = neurogranin

ROS = reactive oxygen species

Tau = taurine

VAD = vitamin A deficiency

VAD_{10} and VAD_{14} = VAD rats at 10 and 14 weeks of experiment

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