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Effect of Vitamin A Deficiency on Retinol and Retinyl Esters Contents in Rat Brain

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Abstract: In the present study, the pattern of vitamin A (retinol and retinyl esters) contents in discrete brain areas was investigated in Wistar rats (both sexes of 10-12 weeks old) fed on vitamin A deficient diet. The animals were placed on standard laboratory diet for the control animals and a vitamin A deficient diet for the experimental animals for 20 weeks. At the end of this period, brain retinol and retinyl esters contents from control and vitamin A deficient diet animals were measured by HPLC. Retinol was the predominant form of retinoids in male rat brains (77 to 92% of total retinol) and retinyl esters were the predominant form in female brain rats (4 to 44% of total retinol). The abundant ester in both sexes was the retinyl linoleate. Olfactory bulb and the midbrain contained the highest quantities of retinol and retinyl esters in both sexes. On the other hand, the vitamin A deficient diet significantly decreased the retinoid contents in male brain, in olfactory bulb (-30.7%), hindbrain (-46.2%) and increased it in forebrain (84.3%) and midbrain (2.2%). Total retinol was decreased in olfactory bulb (-38.7%), forebrain (-44.5%) and midbrain (-30.7%) and increased in hindbrain (23.4%) of vitamin A deficient female rats. In conclusion, retinol and retinyl esters were the brain compounds heterogeneously distributed throughout the brain areas in both the sexes and were significantly affected by vitamin A deficiency status as well.

Key words: Retinol, retinyl esters, olfactory bulb, vitamin A deficiency, sex

INTRODUCTION

Vitamin A (VA) is important micronutrient in the human and the animal diet and is mainly taken up as Retinyl Esters (REs) and retinol (ROH) (Nau and Blaner, 1999).

SRetinoids (VA derivatives) play an important role in several physiological processes such as vision, cellular growth, differentiation, immune response, reproduction including brain functions like hippocampal functions and striatal functions (Lane and Bailey, 2005).

The majority of biological VA effects are mediated through the binding of its active metabolites, all-trans Retinoic Acid (atRA) and 9-cis Retinoic Acid (9cRA) to nuclear receptors. Two types of retinoid nuclear receptors exist: retinoic acid receptors (RAR α, β, γ) and retinoid X receptors (RXR α, β, γ), while RARs have almost equal affinity to atRA and 9cRA, RXRs preferentially bind 9cRA (Chambon, 1996). The RAR are located in the cell nucleus and mediate the activation of specific transcription factors in a model resembling that of steroid/thyroid hormones (Chambon, 1994).

It is well known that Vitamin A Deficiency (VAD) causes night blindness, xerophthalmia, skin keratinization, infertility and immunodeficiency. In brain, the VAD affected all development stages and evoked several malformations (Bavik *et al*., 1996), abnormalities or fail brain development (Maden *et al*., 1998; White *et al*., 2000). In adult brain, the VA deprivation was associated to several dysfunctions: the striatal cholinergic dysfunction (Carta *et al*., 2006), impaired cholinergic transmission (Cocco *et al*., 2002), damaged structure of CA1 neurons, impaired somatostatinergic system (Hernández-pinto *et al*., 2006), reduced amounts of mRNA and protein of neurogranin and neuromodulin in striatum (Etchamendy *et al*., 2003; Husson *et al*., 2004). In spite of these progresses, little is known about of VAD effects.

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An assessment of the VA content should contribute to the understanding of the mechanisms responsible for the production of the various neuropathological changes resulting from VAD. Earlier studies fully detailed the procedure for retinoids detection and analysis in biological samples (Got *et al*., 1995; Barua, 2001; Hartmann *et al*., 2001; Schmidt *et al*., 2002, 2003; Kane *et al*., 2008b). To date, there is no published data on the retinoid content of brains from animals subjected to VAD diet. Present team previously established the ROH and REs contents in whole normal rat brain by HPLC/UV detection (Nasri *et al*., 2005). The present study investigates the detailed neuroanatomical distribution of VA species in the rat brain.

MATERIALS AND METHODS

This study was performed from September 2004 to July 2005 in Department of Biology, Faculty of Science-Kenitra, Ibn Tofail University (animal procedures) and Department of Biology, Faculty of Science and Technique-Mohammadia, University Hassan II (analytical procedures), Morocco.

Care was taken in all laboratory manipulations (sacrifice, removal and dissection samples, extraction and HPLC analysis). Procedures were performed in dark rooms under dim yellow light to prevent photoisomerization and photodegradation.

Animal preparation: Males and females Wistar rats (animal house of Ibn Tofail University, Morocco) were kept on a standard laboratory diet (SNVs.a Fowl Diet, Kenitra, Morocco, composition for 100 g of dry weight: proteins 17.5%; fatty matter 3%; cellulose 6%; sucrose plus maize starch 66%; salts 7.5%; Vit. A 15.000 IU kg^{-1}; Vit. D 3.8 IU kg^{-1}; Vit. E 21.5 mg kg^{-1}) and water *ad libitum* after weaning. Animals were housed in a temperature (22±3°C), light (12 h light/dark cycle, lights on at 8:00) and 55% relative humidity controlled environment. The experiments were conducted in accordance with international guidelines regulating the use of animals for scientific purposes (The Guide for the Care and Use of Laboratory Animals, National Academy of Sciences, USA 1996; The Institute of Laboratory Animal, 1996). Eight rats of either sex received the VAD diet for 20 weeks (composition per 100 g of diet, dry weight: protein: 12.66%; cellulose: 5.2%; hydrogenated sunflower oil: 4.2%; sucrose plus carbohydrate: 68.8%; salt: 4.84%; acetic acid 0.2%; vitamin mixture (lacking VA) 4.1%. This diet had previously been evaluated by Nasri *et al*. (2005). 7 remaining rats of either sex (controls) stayed on the standard laboratory diet.

Body weight, ocular surface and skin characteristics were monitored for signs of VAD.

Chemicals: Standard of all-trans ROH and all-trans retinyl palmitate were a gracefully donation from Hoffman-La Roche laboratories (Basel, Switzerland) and Hoffman-La Roche (Casablanca, Morocco). Hexane, ethanol and butylated hydroxytoluene (BHT) used in the plasma sample preparation procedure were obtained from Merck (Ouameb, Kenitra, Morocco). Chloral hydrate (trichloroacetaldehyde, Cl_3C_2HO) for anesthesia, Sodium chloride (NaCl), Sodium sulphate (Na_2SO_4) and sodium carbonate (Na_2CO_3) with 99% purity for brain retinoid extraction were obtained from Pancrea-quimica (Sochid, Eljadida, Morocco). Methanol, Acetonitril and Dichloromethane (HPLC gradient grade and HPLC eluent), were obtained from Merck and Fluka (Ouameb, Kenitra, Morocco).

Standard solution preparation: Standard solutions were prepared (Nasri, 2007) under yellow light to avoid photo-degradation. The external standardization method was used for retinoid determination in brain. We dissolved 50 mg of all-trans ROH in 100 mL of hexane under ultrasonication to obtain a clear solution. Then, 2.5 mL of the all-trans ROH solution was introduced in a flask, placed in rotavapor for dry evaporation (40°C) and hexane was evaporated using nitrogen. The residue was obtained in a 25 mL of ethanol (1/10 dilution) and optical density was read at 325 nm. We also prepared the standard solution of all-trans retinyl palmitate using similar method as described for all-trans ROH standard solution. Concentrations of all-trans ROH and all-trans retinyl palmitate were UV-spectrophotometrically determined according to the extinction coefficient of the retinoid in ethanol. The working solutions (100 to 2000 μg L^{-1}), which were prepared in ethanol by mixing appropriate amounts of each stock solution, provided a linear-response of UV detection as shown in the regression lines (Fig. 2A, B). These solutions were then stored at -21°C in amber glass vials wrapped in aluminium foil until further use within 1 week.

Brain retinoids extraction and HPLC analysis: After 20 weeks VAD period, the rats were deprived of food for 24 h and then anaesthetized with chloral 7% (7 g kg^{-1}, injected i.p.). Subsequently, animals were sacrificed by decapitation, brains removed and the suitable areas of brain dissected outside on the ice: olfactory bulb, forebrain, midbrain and hindbrain (Fig. 1). These samples of brain were quickly removed, weighed and immediately frozen at -80°C.

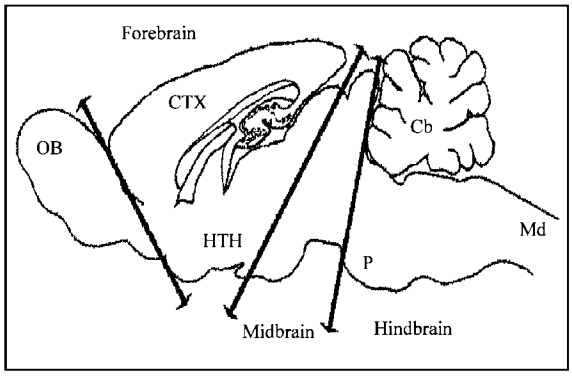

Fig. 1: Diagram of brain areas cutting (Wistar rat). The brain was removed from the skull and under ice was cuted sagittaly and then cutted following black pointers drown above, OB: Olfactory bulb, CTX: Cortex, HTH: Hypothalamus, Cb: Cerebellum, P: Pond, Md: Medulla

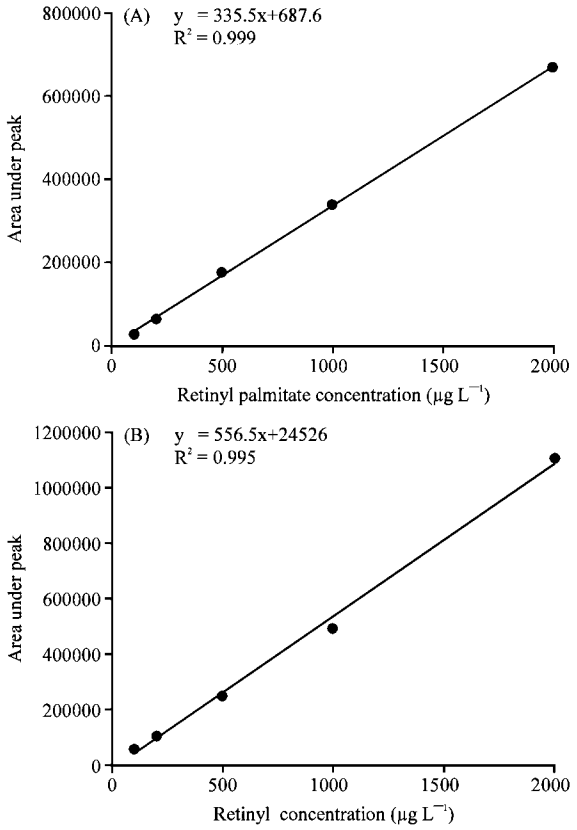

Fig. 2: Calibration curves for (A) retinyl palmitate and (B) retinol. The linear working range is 100-2000 µg L^{-1}

To achieve retinoid extraction and HPLC analysis we pursued the procedure well-described by Nasri *et al.* (2005) and Nasri (2007) with an enhancement of numerous analytical conditions. The nervous tissue fractions (60 mg to 1.2 g) were finely crushed in mortar and homogenized with their double weight of NaCl, Na_2SO_4 and Na_2CO_3 successively (1:1:1, w/w/w). The tissue homogenate must been a powder consistency. The mixture was dissolved with dichloromethane (2 to 12 mL) and vortexed for 5 min and then shaken softly for 10 min manually or using a mechanical shaker. After centrifugation of the mixture (10.000 rpm, 10°C, 15 min, Sigma 4K15-refrigerated), the supernatant was transferred to a drying flask and evaporated at 40°C (rotavapor R-220). We cooled the flask after evaporation with running water and then we dissolved the nervous tissue extract in 600 µL of the following mixture: (Acetonitril/dichloromethane/methanol; 35/20/0.2 mL) and transferred to eppendorf test tube (0.1 to 1 mL). Directly, we injected manually 40 µL of extract in isocratic HPLC system. This system consisted of a Gilson HPLC Model 307 and a Gilson 118 UV detector, regulated at 325 nm. System integrator used for treating chromatograms was an automatic HP model 3395. The Nucleosil RP18 column 5 µm, (240×4, 7 mm) was used for eluting the ROH and REs. The mobile phase was constituted with acetonitril, dichloromethane and methanol proportioned 350/150/4 mL and degassed under ultrasounds. The flow rate was fixed at 1.2 mL min^{-1} and the pressure was optimized from 64 to 67 bar. The limit of detection of the HPLC system used in this task was 0.2 ng.

Plasma retinol extraction and HPLC analysis: Analysis of plasma retinol levels was performed by isocratic phase HPLC according to the method of Nasri *et al.* (2005) and Nasri (2007). Briefly, blood was drawn in moment of decapitation from carotid, collected in recipient contained a drop of heparin (5.000 IU/UI/mL, Leo Pharmaceutical Products, Ballerup, Denmark), centrifuged (Sigma 1-15K refrigerated, 2700 to 3000 rpm) and plasma was separated and stored at -70°C until ROH total analysis by HPLC method with internal standardization. Retinol was extracted from plasma (200 µL) added to 200 µL of internal standard solution (all-trans ROH) into 1 mL of hexane containing of 1% BHT as antioxidant for analysis. Chromatography was performed on a C-18 HPLC column with 100% methanol as the mobile phase (1.2 mL min^{-1}, 109 bar). Retinol was detected by UV absorbance at 325 nm (Hewlett Packard HPLC 1050 System).

Data analysis and calculations: The results are expressed as nanogrames ROH equivalent (Req) per 1 g brain (Means±SEM). Calculus for ROH total was determined according to method of Majchrzak *et al.* (2006) following this equation: ng Req = ng all-trans ROH+ng all-trans

retinyl linoleate/1.92+ng all-trans retinyl oleate/1.92+ng all-trans retinyl palmitate/1.83+ng all-trans retinyl stearate/1.93. The calculation of the conversion factors (1.83; 1.92; 1.93) was done by dividing the molecular weight of RE by molecular weight of ROH. The statistical evaluation, to determine differences between VA contents of brain from male and female rat, was done by employing the t-test. Differences were regarded as statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Vitamin A status monitoring: Figure 3A and B show the effect of 20 weeks off food consumption in VAD and control rats. The curves demonstrated a significant reduction in body weight gain in rats fed a VAD diet as compared with control rats. The average difference between body weights was 111.2±13.07 g in males ($p<0.001$, by student t-test) and 54.38±4.48 g in females ($p<0.001$, by student t-test). This reduction of body weight was maintained until end of experiment for male rats.

VAD rats of both sexes displayed a hair loss appeared between 12 and 20 weeks after initiation of VAD feeding (nape and throat bread in females, bread of caudal region and bordering sexual organs in males) and did not exhibit any sign of blindness.

The plasma ROH concentration significantly decreased in VAD rats (-88.6%, $p<0.01$). The control rats ($n = 14$) had 2.72 0.12 μmol L^{-1}, where as the VAD rats ($n = 16$) had 0.31±0.07 μmol L^{-1}. Values are the Mean±SD. The difference between sexes did not determine in this analysis.

Standardization and linearity: Figure 2 shows representative calibration curves for at ROH and at PAL. These standard solutions were assayed at five concentrations in the range of 100 to 2000 μg L^{-1}. Each point represents either two or three replicates and R^2 values are greater than 0.99. The data indicates good linearity of this method. Figure 4A-D show standards chromatograms (all-trans ROH and all-trans retinyl palmitate) and forebrain fraction chromatograms.

Neuroanatomical contents of retinol and retinyl esters: Figure 5A-P show HPLC chromatograms of retinoid extracted from rat brain areas. HPLC analysis length was 10 min when all retinoid studied were eluted. The contents obtained from brain areas of rat samples are shown in Table 1, when the total ROH expressed by ng ROH equivalent per 1 g of brain area (ng Req g^{-1}).

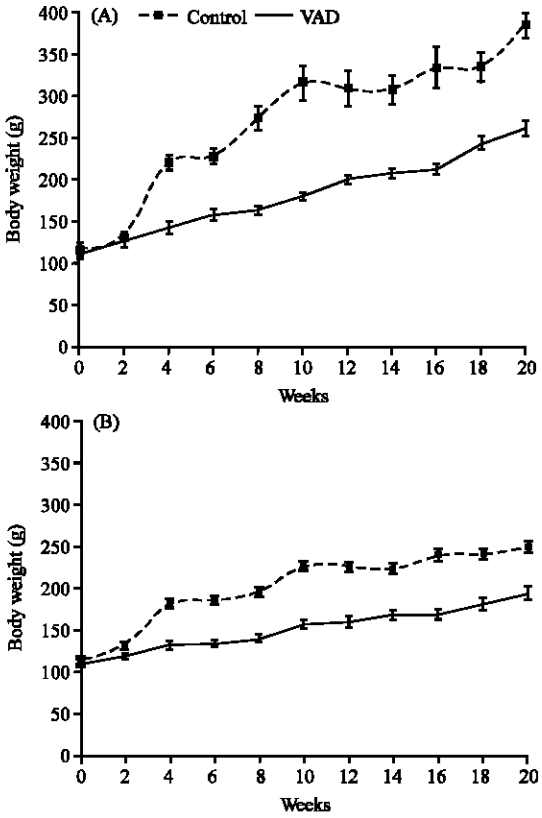

Fig. 3: Effect of VA deficient diet on body weight. Each point is the mean±SEM of seven or eight experiments: Points correspond to control rats (---) or rats fed a VA deficient diet (—). Statistical comparison versus control: ***$p = 0.001$. (A) the graph for male rats and (B) the graph for female

Retinoid were highly represented in male brain rat ($p<0.001$, Table 1). The ROH was the predominant retinoid form in male brain in opposition with REs in female brain rat (Table 2). Moreover, REs (linoleate, oleate, palmitate and stearate) had the heterogeneous distribution across discrete brain areas, which retinyl-linoleate was the major REs in brain rat (Table 2).

VAD induced a decrease and an increase of retinoid contents in brain areas. In male rat, the t-test indicated a significant retinoid reduce in olfactory bulb ($p<0.01$) and hindbrain ($p<0.01$) and an increase in forebrain ($p<0.01$) and midbrain. In female rat, VAD decreased the retinoid in olfactory bulb, forebrain and midbrain ($p<0.01$) (Table 3) excepting the hindbrain when we observed a significant increase ($p<0.05$).

Finally, we established widely the significant difference of retinoid contents between sexes in whole brain areas (Fig. 6A, B).

Table 1: Retinol and retinyl esters contents in brain areas of both sexes expressed by ng Req g^{-1}

	Olfactory bulb		Forebrain		Midbrain		Hindbrain	
	Control	VAD	Control	VAD	Control	VAD	Control	VAD
Males								
Total retinol[a]	5960.60± 71.40	4127.74±101.03	193.00±5.02	355.72±5.52	5120.66±85.88	5235.01±0.860	452.38±4.24	243.54±9.71
Retinyl esters[b]	1334.68± 55.72	1014.06±21.14	62.74±7.09	87.49±9.86	587.46±47.61	495.45±10.49	33.87±5.08	35.31±4.14
Retinol	4625.92± 15.69	3113.68±79.89	130.26±2.07	268.23±5.66	4533.21±38.27	4739.57±11.34	418.51±9.32	208.23±5.57
Retinyl linoleate[c]	920.07±22.510	687.70±4.23	26.99±0.53	26.60±0.19	411.90±9.930	341.84±6.760	33.87±5.08	35.31±4.14
Retinyl oleate[d]	328.45±14.330	181.18±3.66	4.46±0.84	5.62±2.83	142.81±21.28	136.62±7.700	<d.l.	<d.l.
Retinyl palmitate[e]	86.17±18.890	145.11±29.04	21.83±8.71	34.68±0.11	29.49±13.15	16.99±3.970	<d.l.	<d.l.
Retinyl stearate[f]	<d.l.	<d.l.	9.46±2.98	20.59±12.39	3.26±3.260	<d.l.	<d.l.	<d.l.
Females								
Total retinol[a]	1369.92±6.83	839.94±14.33	127.40±17.52	70.76±9.56	288.80±15.64	200.16±19.43	111.62±18.22	137.69±16.13
Retinyl esters[b]	1315.33±6.83	797.59±14.33	90.19±11.56	55.43±9.72	208.47±3.750	183.91±19.43	63.56±12.02	130.09±16.13
Retinol	54.59±0.05	42.35±0.030	37.21±5.960	15.33±0.16	80.33±11.89	16.25±0.010	48.07±6.200	7.61±0.070
Retinyl linoleate[c]	838.64±8.84	486.65±9.900	22.37±4.760	13.44±4.38	109.79±0.490	90.81±0.830	28.31±2.790	71.45±2.400
Retinyl oleate[d]	301.32±5.44	207.56±4.570	9.36±2.650	7.09±2.58	40.69±0.040	38.14±0.410	12.21±1.430	24.11±0.340
Retinyl palmitate[e]	107.94±15.76	54.55±31.40	38.85±4.040	23.57±2.69	38.50±1.190	29.96±21.08	17.26±5.950	20.24±12.28
Retinyl stearate[f]	67.43±5.35	48.82±2.600	19.61±0.110	11.33±0.07	19.49±3.010	24.99±0.410	5.78±1.840	14.29±1.110

[a]Total retinol ng Req g^{-1}: ng retinol+ng retinyl linoleate/1.92+ng retinyl oleate/1.92+ng retinyl palmitate/1.83+ ng retinyl stearate/1.93. [b]Retinyl esters ng Req g^{-1}: [c]ng retinyl linoleate/1.92+ [d]ng retinyl oleate/1.92+ [e]ng retinyl palmitate/1.83+ [f]ng retinyl stearate/1.93. d.l.: Detection limit 0.2 ng, VAD: Vitamin A deficient rats (n = 8), Control (n = 7)

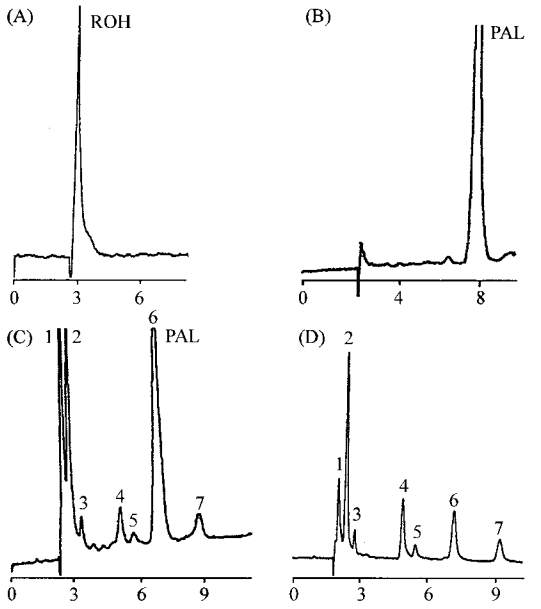

Fig. 4: Chromatograms of standard solutions (A) ROH: Retinol, (B) PAL: Retinyl palmitate (16:0) and typical chromatograms of forebrain fraction of Wistar rat with internal standard solution, (C) (100 mL of forebrain fraction added to 200 mL of at PAL stock standards solutions working) and without internal standard solution (D). Conditions: column. 25 cm × (240×4, 7 mm) i.d.; stationary phase, silica, 5 μm ; mobile phase 1.2 mL min^{-1} Acetonitril, dichloromethane and methanol proportioned 350/150/4 mL; pressure: 64 bar, UV detector, 325 nm. Peaks: 1: mobile phase peak, 2: biological matrix, 3: retinol, 4: retinyl linoleate, 5: retinyl oleate, 6: retinyl palmitate and 7: retinyl stearate

In the present study, animals were fed a VAD diet for 20 weeks. Following this period, the rats showed clear signs of VAD such as hair loss, conjunctival epithelium damaged and a reduction in body weight gain with respect to control rats, as described by Cocco *et al.* (2002) and Hernández-Pinto *et al.* (2006). Moreover, the retinoid plasma concentration was decreased significantly (-88.6%) in according with data reported by Carta *et al.* (2006).

In addition, in both control and VAD diet, we demonstrated that the retinoid content is greater in the male brain in comparison with female brain. To date, the sex differences of retinoid brain contents has not been explained. This study illustrates the divergence of retinoid brain distribution between sexes in rats. Several aspects of differences existing at the physiological and biochemical level between sexes had previously been investigated: Rosa-Molinar *et al.* (1997) established the high endogenous Retinoic Acid (RA) levels in fish spinal cord of immature males compared with immature females, Martini and Murray (1993) described a sex-related difference in liver metabolism of all-trans-RA in Wistar rats (male>female) and finally, Marchetti *et al.* (1997) found that in rats, the liver metabolism of RA isomers varies with sex. Differences between sexes seem to be governed by sex steroids and other hormones as demonstrated in human by the correlation between sex serum hormones and retinol concentrations over menstrual cycle in adolescent girls (Barbin *et al.*, 2004). Additionally, we also showed the predominance of ROH form in male brain rat (67 to 92%) compared to female rat brain (4 to 43.1%). Werner and Deluca (2002) demonstrated that in male Sprague-Dawley rats the RA is not transported preferentially to the brain but is likely to be synthesized there more efficiently than in other target

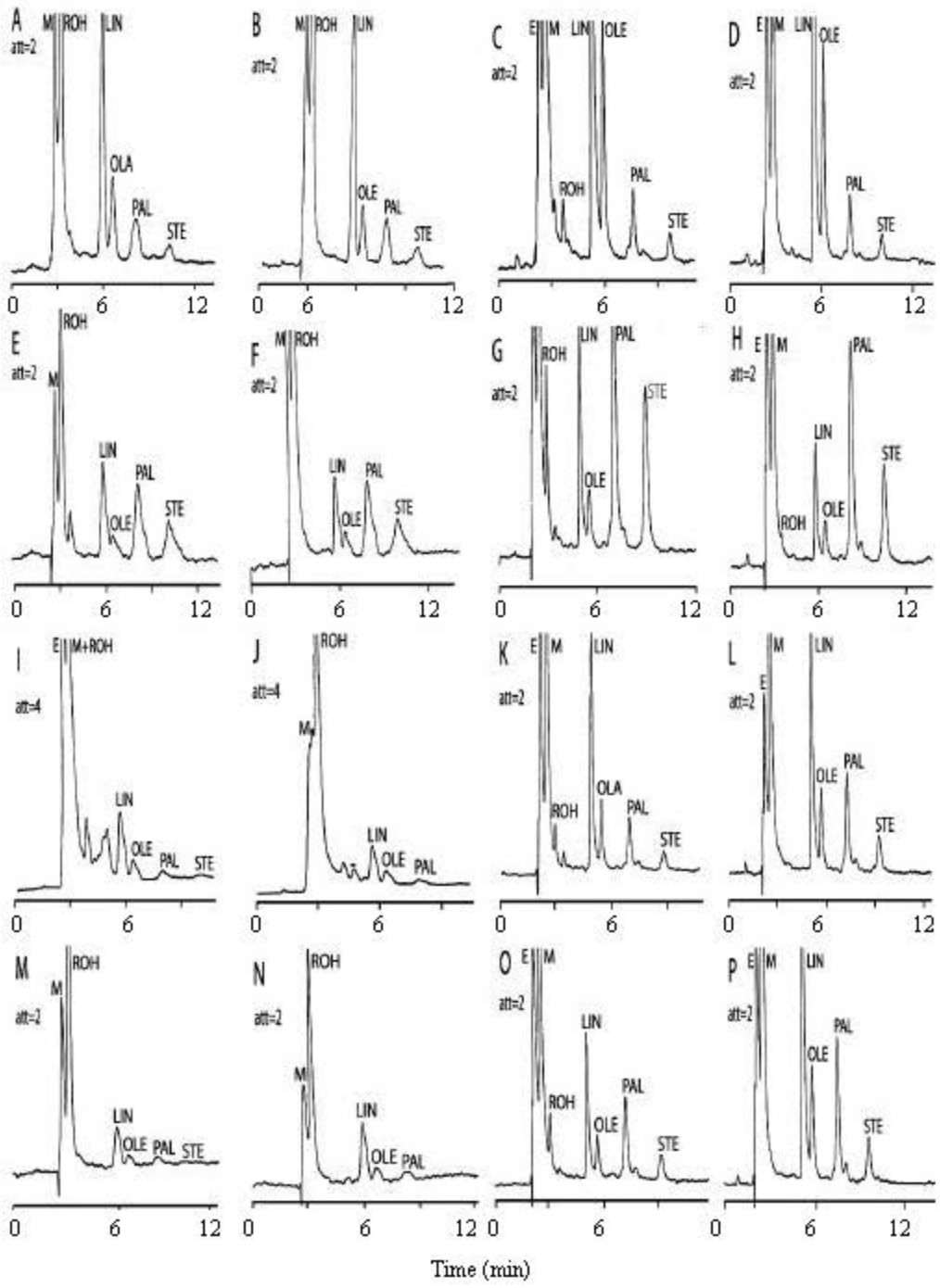

Fig. 5: High-performance liquid chromatograms of fractions obtained for rat brain areas. Olfactory bulb (A, B, C, D), forebrain (E, F, G, H), midbrain (I, J, K, L) and hindbrain (M, N, O, P); males (A, E, I, M: controls and B, F, J, N: vitamin A deficient rats) and females (C, G, K, O: Controls and D, H, L, P: vitamin A deficient rats). Conditions: column. 25 cm × (240×4, 7 mm) i.d.; stationary phase, silica, 5 m ; mobile phase 1.2 mL min^{-1} Acetonitril, dichloromethane and methanol proportioned 350/150/4 mL; pressure: 64 to 67 bar, UV detector, 325 nm. Peaks: E: Mobile phase peak, M: Biological matrix, ROH: Retinol, LIN: Retinyl linoleate, OLA: Retinyl oleate, PAL: Retinyl palmitate, STE: Retinyl stearate. (att): Attenuation, all chromatogram in attenuation 2 except I and J were in attenuation 4

Table 2: Composition (%) of retinol and retinyl esters in the different brain areas

	Olfactory bulb		Forebrain		Midbrain		Hindbrain	
	Control	VAD	Control	VAD	Control	VAD	Control	VAD
Males								
Retinol	77.6	75.5	67.5	75.4	87.0	90.5	92.0	85.8
Retinyl linoleate	15.4	16.7	14.0	7.5	7.9	6.5	7.4	14.5
Retinyl oleate	5.5	4.4	2.3	1.6	2.7	2.6	0.7	0.0
Retinyl palmitate	1.4	3.5	11.3	9.7	0.6	0.3	0.0	0.0
Retinyl stearate	0.0	0.0	4.9	5.8	0.1	0.0	0.0	0.0
Females								
Retinol	4.0	5.0	29.2	21.6	27.8	8.1	43.1	5.5
Retinyl linoleate	61.2	57.9	17.6	19.0	38.0	45.4	25.4	51.9
Retinyl oleate	22.0	24.7	7.3	10.0	14.1	19.1	10.9	17.5
Retinyl palmitate	7.9	6.5	30.5	33.3	13.3	15.0	15.5	14.7
Retinyl stearate	4.9	5.8	15.4	16.0	6.7	12.5	5.2	10.4

VAD: Vitamin A deficient rats (n = 8), control (n = 8)

Table 3: Effect of vitamin A deficient diet (%)* on vitamin A contents

	Olfactory bulb	Forebrain	Midbrain	Hindbrain
Males				
Total retinol	-30.7	84.3	2.2	-46.2
Retinyl esters	-24.0	39.4	-15.7	-38.5
Retinol	-48.6	51.4	4.4	-101.0
Retinyl linoleate	-25.2	-1.5	-17.0	4.2
Retinyl oleate	-44.8	26.0	-4.3	-
Retinyl palmitate	68.4	58.9	-42.4	-
Retinyl Stearate	-	117.7	-100.0	-
Females				
Total retinol	-38.7	-44.5	-30.7	23.4
Retinol	-28.9	-142.7	-394.3	-531.7
Retinyl esters	-39.4	-38.5	-11.8	104.7
Retinyl linoleate	-42.0	-39.9	-17.3	152.4
Retinyl oleate	-31.1	-24.2	-6.3	97.5
Retinyl palmitate	-49.5	-39.3	-22.2	17.2
Retinyl Stearate	-27.6	-42.2	28.3	147.3

*Reduce or enhance percentage of brain vitamin A contents in rats fed vitamin A deficient diet, % = [(ng of retinoid in VAD rats−ng of retinoid in control)/ ng of retinoid in VAD rats]×100

tissues. It is known that ROH is the major retinoid transported in the bloodstream. MacDonald *et al.* (1990) reported significant movement of ROH across the blood-brain barrier. Thus, in the male rat, the high content of ROH throughout the brain results probably from the high efficiency ROH transport across the blood-brain barrier. This was also shown by Werner and Deluca (2001) in VAD male rats.

In present study, the female rat brain (control and VAD) contained the high REs as dominant form of retinoid brain. This is in disagreement with earlier reports where ROH levels were determined at 0.65±0.44 μg g^{-1} of ROH (equivalent to 2.72±1.54 nmol g^{-1}) and 0.52±0.30 nmol g^{-1} of REs in adult female NMRI mice receiving a 15,000 IU VA kg^{-1} in diet (Schmidt *et al.*, 2002, 2003). Thus, the mice female brain presented high ROH content compared to REs. The several anatomic, physiologic, biochemical and genetic differences between species seem to be the reason for the distinction of their retinoid brain distribution. The retinoid brain contents are not the conservative physiological parameters, such as

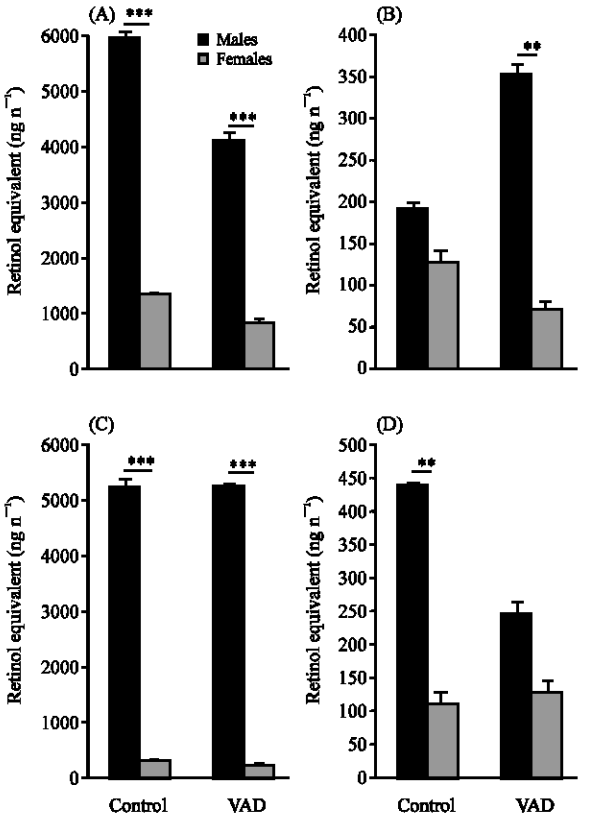

Fig. 6: Levels of retinol equivalent (REq) in different brain areas. Differences between males and females in control or vitamin A deficient rat (VAD) were noted. *p<0.05, **p< 0.01, ***p<0.001. (A) olfactory bulb, (B) forebrain, (C) midbrain and (D) hindbrain. The contents were expressed in ng REq per 1 g brain area (Mean±SEM)

body temperature (36-38°C) hematocrit (40-45%) and serum albumin concentration (3-4 g dL^{-1}) which are relatively conserved among species and are independent of animal size (Davies and Morris, 1993). Other finding

demonstrated this concept: The mouse serum retinol varied from 0.17 to 0.81 nmol mL^{-1} (Schmidt *et al*., 2002; Kane *et al*., 2008a) while the rat serum retinol varied from 0.14 to 2.34 nmol mL^{-1} (Furr and Olson, 1988).

In this study, we also demonstrate that the olfactory bulb and midbrain (normal and VAD) contain the highest levels of retinoids for both sexes. The highest RA precursor contents (ROH and RE) in olfactory bulb may explain by distinguished retinoid signalling demonstrated in olfactory bulb. Wagner *et al*. (2002) reported that the olfactory bulb is the RA-richest part of the adult brain, where an intense RA signal was noted in olfactory glomerular layer. Moreover, RA induced transcriptional activation in olfactory granular and glomerular layers in the subventricular zone (SVZ)-olfactory bulb pathway of the adult rodent (Thompson *et al*., 2002). In SVZ, which generates the new neurons destined for the olfactory bulb, ROH was shown to have similar effect (Doetsch *et al*., 1999a, b; Stenman *et al*., 2003; Wang *et al*., 2005). This study reported that ROH was in high contents in olfactory bulb, thus it played relatively two roles: like RA, a regulator and a modulator of genes controlling the olfactory bulb functions and like circulating form which could be distributed easily to sites where RA synthesis is needed. The midbrain is an essential site of dopamine production (localised in the substantia nigra compacta and ventral tegmental area). Interestingly, key components of retinoid signalling, including retinaldehyde deshydrogenase, RARs and RA binding proteins, are found in the dopaminergic pathways (Krezel *et al*., 1998). This is consistent with our data found the high retinoid content in midbrain especially in male brain. The high presence of retinoid in midbrain allowed a normal processing of locomotion and dopamine signalling. The RARβ/RXRβ, RARβ/RXRγ and RXRβ/RXRγ double null mutant mice presented a low forward locomotion and rearing frequency in the open field test. Also, motor coordination tested by performance on the rotarod in the same double null mutant mice demonstrated quickly the fell indicating impaired performance (Krezel *et al*., 1998). Furthermore, the expression of dopamine D1 and D2 receptors or D2 receptor mRNA expression was decreased 40% in the striatum following the absence of some isotypes like in RARβ/RXRβ, RARβ/RXRγ RXRβ/RXRγ and RARα/RXRγ null mice mutants (Samad *et al*., 1997; Krezel *et al*., 1998).

We demonstrate that retinyl linoleate is the most presented form of REs in male and female brain excluding forebrain when it and retinyl palmitate existed at proximate levels. This suggests that retinyl linoleate has an important role to play in the brain. Retinyl palmitate was the VA predominant form in other tissues of mammals like liver (Majchrzak *et al*., 2006) and skin (Ribaya-Mercado *et al*., 1994). The brain had a special picture of REs distribution and the reason of retinyl linoeate storage as favourite brain REs was not clear by simple quantification. The advanced experiments required to answer why retinyl linoleate was the predominant in brain rat if the literature had absent in the field.

In addition, it is known that RE levels vary in different brain region, as do ROH levels (Barua and Furr, 1998; Kane *et al*., 2008a), which is compatible with the heterogeneous distribution of different esters (linoleate, oleate, stearate and palmitate) across distinct brain areas as presented in this study. We pointed to a regional decrease or increase in ROH contents caused by VAD diet. Additionally, RE contents also declined or increased in brain rat following VAD diet.

The ROH and REs were the brain compounds distributed heterogeneously through brain rat areas, sex-dependent and were affected significantly by a VAD status.

Present data were according partially with data imported by Liu and Gudas (2005), who demonstrated that ROH content in brains of mice receiving a VAD diet decreased significantly while RE content remained unchanged.

In conclusion, we established the highest retinoid contents in male brain rat are related to higher ROH content. Thus, ROH is the predominant form of brain retinoids in male rats whereas it is REs in the female rat brain. Furthermore, the olfactory bulb and the midbrain were the richest brain areas and linoleate was the most represented retinyl ester in the rat brain. Finally, VAD affected the retinoid distribution of rat brain and with the great homeostasis, the retinoid controlled their pathways when the VAD installed.

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