Archives of Biochemistry and Biophysics 604 (2016) 121–127

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Age-related changes in retinoic, docosahexaenoic and arachidonic acid modulation in nuclear lipid metabolism

Virginia L. Gaveglio, Ana C. Pascual, Norma M. Giusto, Susana J. Pasquaré*

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form
8 June 2016
Accepted 24 June 2016
Available online 26 June 2016

Keywords:
Diacylglycerol
Monoacylglycerol
Nuclei
Retinoic acid
Docosahexaenoic acid
Aging

ABSTRACT

The aim of this work was to study how age-related changes could modify several enzymatic activities that regulate lipid mediator levels in nuclei from rat cerebellum and how these changes are modulated by all-trans retinoic acid (RA), docosahexaenoic acid (DHA) and arachidonic acid (AA). The higher phosphatidate phosphohydrolase activity and lower diacylglycerol lipase (DAGL) activity observed in aged animals compared with adults could augment diacylglycerol (DAG) availability in the former. Additionally, monoacylglycerol (MAG) availability could be high due to an increase in lysophosphatidate phosphohydrolase (LPAPase) activity and a decrease in monocylglycerol lipase activity. Interestingly, RA, DHA and AA were observed to modulate these enzymatic activities and this modulation was found to change in aged rats. In adult nuclei, whereas RA led to high DAG and MAG production through inhibition of their hydrolytic enzymes, DHA and AA promoted high MAG production by LPAPase and DAGL stimulation. In contrast, in aged nuclei RA caused high MAG generation whereas DHA and AA diminished it through LPAPase activity modulation. These results demonstrate that aging promotes a different nuclear lipid metabolism as well as a different type of non-genomic regulation by RA, DHA and AA, which could be involved in nuclear signaling events.

1. Introduction

Nuclear lipids have been detected not only in the nuclear membrane but also in the nuclear matrix and chromatin [1,2]. The enzymes related to their metabolism generate several lipid messengers in the nucleus which appear to be involved in signaling processes. Nuclear events such as DNA stabilization, nuclear receptor activation, transcription factors and RNA and DNA polymerase activities, could be modulated by lipids [3]. Thus, lipid signaling in nuclei could have a key role in physiological as well as pathological events. Many studies have reported that aging, neurodegenerative processes and various neurological disorders are accompanied by modifications in the composition and metabolism of glycerophospholipids, sphingolipids, and cholesterol [4,5]. Previous work carried out by our research group on different brain areas of aged rats demonstrated changes in lipid profile and metabolism with respect to adults. Some of these changes consisted in a different regulation of the enzymatic activities involved in DAG and phosphatidic acid (PA) production [6,7]. In addition, it was shown that insulin regulates PA/DAG balance in cerebral cortex synaptosomes and that this regulation also changes in aged animals [8]. As regards nuclear lipid metabolism, we have demonstrated active PA metabolism in isolated nuclei from rat cerebellum which involves LPPs, DAGL and monocylglycerol lipase (MAGL) enzymatic activities as well as PLA and LPAPase activities in neural and non-neural tissue [9,10]. As with other areas of the central nervous system (CNS), both the anatomy and functions of the cerebellum

Abbreviations: 2-AG, 2-arachidonoylglycerol; BSA, bovine serum albumin; DAG, diacylglycerol; DAGL, diacylglycerol lipase; DAPI, 4′,6-diamidino-2-phenylindole; DMSO, Dimethyl sulfoxide; DTT, dithiotreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis (b-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra acetic acid; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPAPase, lysophosphatidate phosphohydrolase; LPP, lipid phosphate phosphatase; MAG, monoacylglycerol; MAGL, monoacylglycerol lipase; MOPS, 3-(N-morpholino) propane sulfonic acid; NEM, N-ethylmaleimide; PA, phosphatidic acid; PPAR, peroxisome proliferator-activated receptor; PC, phosphatidylcholine; PA-PLA, phosphatidic acid-phospholipase A; PUFA, polyunsaturated fatty acid; RA, all-trans retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; TLC, thin-layer chromatography.

* Corresponding author. Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), CONICET-Bahía Blanca and Universidad Nacional del Sur (UNS), Edificio E1, Camino La Carrindanga km 7, 8000 Bahía Blanca, Argentina.
E-mail address: pasquare@criba.edu.ar (S.J. Pasquaré).

http://dx.doi.org/10.1016/j.abb.2016.06.017
0003-9861/

have been observed to undergo alteration in neurodegenerative processes such as Alzheimer disease and aging [11,12]. It has been shown that among other enzymes and transcription factors, RA modifies the activities of nuclear phospholipases through a non-genomic mechanism [13]. RA has a critical role in the development of CNS and in neural plasticity [14] and also appears to be involved in neuroprotective mechanisms [15,16]. In agreement with this, a decrease in RAR expression and in its signaling in aged animals has been related to cognitive decline, which could be reversed by RA treatment [17]. Interestingly, previous studies have demonstrated that RA could also bind to and activate peroxisome proliferator-activated receptors (PPARs) although their classical ligands are polyunsaturated fatty acids (PUFA) and eicosanoids [18]. PPAR activation is also associated with neuroprotection [16]. In addition, many PPAR ligands were observed to have the ability to regulate MAPK activation, transcription factors or enzymatic activities in a non-genomic way [19]. Among polyunsaturated fatty acids (PUFA), DHA has also been postulated as a neuroprotective lipid with a significant role in either attenuating or preventing neurodegenerative processes by multiple mechanisms [20,21].

Based on this background, the aim of the present research is to study age-related changes in enzymatic pathways involved in PA/DAG/MAG metabolism in isolated nuclei from rat cerebellum as a CNS model. Our research also focuses on the regulation of this nuclear metabolism by neuromodulators such as RA and PUFA, enabling us to analyze the effects of age-associated neurodegeneration on nuclear lipid metabolism and its regulation by neuroprotection-related molecules.

2. Materials and methods

2.1. Materials

[2−^{3}H]Glycerol (200 mCi/mmol) was obtained from New England Nuclear-Dupont (Boston, MA, USA). Pre-blended dry fluor 2a70 (98% PPO and 2% bis-MSB) was obtained from Research Products International Corp. (Mount Prospect, IL, USA). The detergent ARKOPAL N-100 was obtained from *ALSI Química Sanitaria* (La Plata, Buenos Aires, Argentina). 2-arachidonoylglycerol [glycerol-1,2,3-^{3}H] (40 Ci/mmol), unlabeled 2-arachidonoylglycerol, and lysophosphatidic acid, 1-oleoyl [oleoyl-9,10-^{3}H(N)] (54 Ci/mmol) were obtained from American Radiolabeled Chemicals, Inc. (Saint Louis, MO, USA). Oleoyl-L-α-lysophosphatidic acid, fatty acid-free bovine serum albumin (BSA) and Palmitoyl-L-α-lysophosphatidylcholine acid (LPC) were obtained from Sigma-Aldrich (St. Louis, MO, USA). GSK 0660 was purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA). All the other chemicals used were of the highest purity available.

2.2. Purified nuclear fraction preparation

Wistar-INIBIBB stock adult (4 months old) and aged (24–28 months old) rats were kept under constant environmental conditions and fed on a standard pellet diet. All procedures were carried out following the guidelines issued by the Animal Research Committee (Resolution N° CDBByF 791/14) of the *Universidad Nacional del Sur* (Argentina) in accordance with the Guide of the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research (ILAR) of the National Academy of Science (Bethesda, MD, USA). Adult and aged rats were killed by decapitation and the cerebellum was immediately dissected. The nuclei isolation procedure was followed as described elsewhere [9]. The purified nuclear fraction was stored at −70 °C until used and re-suspended in a buffer that preserves nuclear integrity (50 mM Tris-HCl buffer, pH 7.5, containing 25 mM KCl and 5 mM $MgCl_2$) to perform the enzymatic assays. The purity of nuclear preparations was assessed as previously described [9,10].

2.3. Preparation of radioactive 1, 2-diacyl-sn-glycerol-3-phosphate and 1, 2-diacyl-sn-glycerol and procedure of enzymatic assays

Radioactive PA and DAG were obtained from [2−^{3}H]glycerol-PC ([^{3}H]PC) which was synthesized from bovine retinas incubated with [2−^{3}H]glycerol (200 mCi/mmol) as previously described [9,22]. [^{3}H]PA and [^{3}H]DAG specific activities were calculated by measuring phosphorous or fatty acid content of an aliquot of each substrate, respectively [9].

LPP, DAGL, MAGL, LPAPase, and PA-PLA assays were conducted as previously specified [9,10]. LPP and DAGL enzymatic reactions were stopped by adding chloroform:methanol (2:1, v/v), MAGL enzymatic reaction was stopped by adding chloroform:methanol (1:1,v/v), and LPAPase and PA-PLA assays were stopped by adding chloroform:methanol:HCl 0.1 N (2:1:0.025, v/v/v). The blank preparations were identical to that of each enzymatic assay except that the membrane fraction was either boiled for 5 min or inactivated by the addition of chloroform:methanol (2:1, v/v) before use.

Lipids were extracted according to Folch [23] and chromatographed by TLC on silica gel G or H plates using different developing solvents as previously described [9,24]. Chromatograms were visualized by exposure to iodine vapors and the areas corresponding to LPA, PA, MAG and DAG were scraped off for counting by liquid scintillation. The aqueous phase from Folch extraction containing radiolabel water soluble products (glycerol or glycerol 3-phosphate) was concentrated to dryness and counted by liquid scintillation. Radiolabel samples were counted after the addition of 0.5 ml water and 10 ml of 5% pre-blended dry fluor 2a70 in toluene/detergent (4/1, v/v).

2.4. Experimental design

For analysis of RA (10 μM), DHA (10 μM) and AA (10 μM) effects, each one was added simultaneously with the substrate corresponding to each enzymatic activity and incubated for 60 min. To determine whether DHA and AA action were mediated either totally or partly by PPARs, pre-incubations were carried out for 10 min in the presence of a selective PPARs antagonist (GSK 0660, 10 μM) prior to substrate or substrate plus DHA or AA additions. RA, DHA and AA were prepared as stock solutions in ethanol. The dilutions used in the assays were prepared at the moment of the experiments. The ethanol in the assays was used at a final concentration (0.1%) that did not affect the enzymatic activities.

2.5. Other methods

Protein and lipid phosphorus were determined according to Lowry [25] and Rouser [26], respectively.

2.6. Statistical analysis

All data are given as means ± SD of 3 replicates of a minimum of 2 different experiments. Experiments were performed in samples obtained from different pools of animals (ten animals per pool). Student's t-tests were performed for comparisons between aged and adult rats and analysis of variance (ANOVA) with Tukey's post-test for multiple comparisons, using GraphPad software version 5.00 (San Diego, California, USA, www.graphpad.com).

3. Results

Age-related changes in the enzymatic pathways involved in PA/

DAG/MAG metabolism in rat cerebellum nuclei.

Data shown in Fig. 1 indicate LPP/DAGL, LPAPase, PA-PLA, DAGL and MAGL enzymatic activities in the isolated nuclear fraction from the cerebellum of adult and aged rats. It can be observed that DAG production by LPPs increased by 42% ($p < 0.05$) in aged rats whereas MAG production by DAGL coupled to LPPs was found to remain unmodified (Fig. 1 A). In addition, MAG production increased by 78% ($p < 0.005$) by LPAPase activity (Fig. 1 B) whereas its production by DAGL action was diminished by 35% (Fig. 1 D, $p < 0.05$) in aged animals with respect to adults. MAGL activity was inhibited by 16% (Fig. 1 E, $p < 0.005$) as glycerol production was lower in aged nuclei. Moreover, PA-PLA activity was found to decrease by 43% ($p < 0.001$) as shown in Fig. 1C.

Effect of RA on PA/DAG/MAG metabolism in adult and aged rat cerebellum nuclei.

RA treatment of isolated nuclei produced significant changes in the enzymatic activities assayed (Fig. 2). LPP (Fig. 2A) and LPAPase (Fig. 2B) activities remained unmodified whereas a 30% ($p < 0.005$) inhibition was detected in PA-PLA activity in adult animals (Fig. 2C). Important changes were also observed in DAGL and MAGL activities, which were inhibited by 54% ($p < 0.001$) and 31% ($p < 0.001$), respectively (Fig. 2D and E). RA only modified LPAPase activity in isolated nuclei from aged cerebellum as indicated by the 31% increase ($p < 0.001$) in MAG formation (Fig. 2B).

Effect of AA and DHA on LPP, LPAPase and PA-PLA activities in adult and aged rat cerebellum nuclei.

When the action of AA and DHA was assessed in isolated nuclei our results showed that neither AA nor DHA modulated LPP activity in adult (Fig. 3A) and aged cerebellum nuclei (Fig. 3B). Interestingly, a slightly DHA-increasing effect (15%, $p < 0.05$) on LPP activity was observed when PPARs were antagonized by GSK 0660 in aged nuclei (Fig. 3 B). LPAPase activity was stimulated by AA (22%, $p < 0.05$) and DHA (41%, $p < 0.005$) (Fig. 3C) in adult cerebellum nuclei, and an inhibition (50%, $p < 0.005$) by both fatty acids in LPAPase activity was observed (Fig. 3D) in aged nuclei. In contrast to our observations in adults, the effect of AA and DHA on enzymatic activity was reversed in aged nuclei, reaching values similar to those of controls when PPARs were blocked by GSK0660 (Fig. 3D). DHA led to a 44% ($p < 0.005$) and 57% ($p < 0.001$) PA-PLA activity decrease in adult (Fig. 3E) and aged nuclei (Fig. 3F), respectively, whereas AA exerted a similar inhibitory effect (43%, $p < 0.005$) only in aged nuclei (Fig. 3F). Additionally, a major diminution by DHA and AA was observed when GSK 0660 was used both in adult (70%, $p < 0.005$, Fig. 3E) and aged (95%, $p < 0.001$, Fig. 3F) nuclei.

Effect of AA and DHA on DAGL and MAGL activities in adult and aged rat cerebellum nuclei.

Whereas both fatty acids produced a similar increase in DAGL activity (69%, $p < 0.001$) (Fig. 4A) in adult rats, this activity remained unmodified in aged rats (Fig. 4B). PPAR antagonist decreased DAGL activity by 84% ($p < 0.001$) and by 56% ($p < 0.005$) in adult (Fig. 4A) and aged (Fig. 4B) rats, respectively. The antagonist effect remained unmodified even after the addition of fatty acids (Fig. 4A and B). Neither AA nor DHA modified 2-AG hydrolysis through MAGL activity in adult (Fig. 4C) and aged (Fig. 4D) cerebellum nuclei. PPAR antagonist alone reduced this activity by 28% ($p < 0.005$) and 46% ($p < 0.005$) in adult (Fig. 4C) and aged (Fig. 4D) nuclear preparations, respectively. Interestingly, only the addition of DHA reversed the GSK 0660 effect, reaching control levels in adult ($p < 0.005$) (Fig. 4C) and aged ($p < 0.001$) (Fig. 4D) nuclei.

4. Discussion

Aging is a physiological process during which important changes occur in membrane lipid composition and metabolism, especially in the neural tissue [6,27]. The changes in LPP and DAGL enzymatic activities lead to a higher nuclear DAG availability in cerebellar nuclei from aged animals with respect to adults. This is strengthened by a marked decrease in the production of LPA from PA by PA-PLA action, which may favor PA metabolism through LPP activity. Likewise, an increase in DAG formation from PC by PLC activity has also been detected in isolated nuclei from the liver of aged rats [28]. The fact that MAGL and LPAPase activities were observed to be respectively inhibited and stimulated in aged rats is indicative of higher MAG availability. These changes in enzymatic activities in aged rats could be related not only to nuclear lipid remodeling but also to a particular intra-nuclear signaling involved

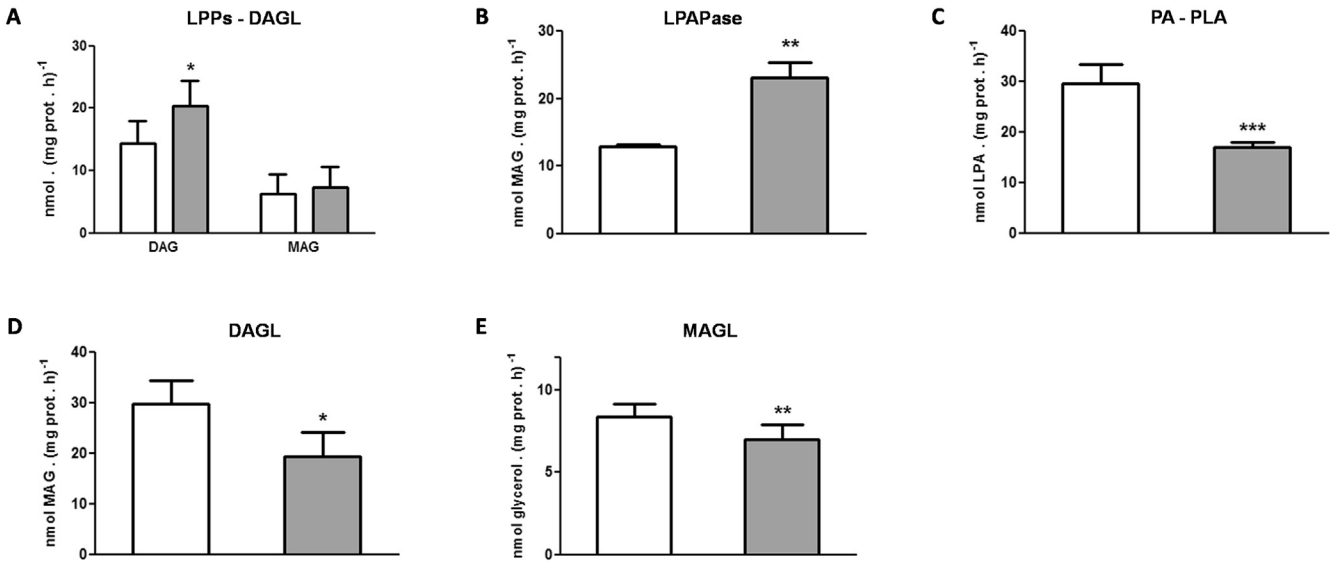

Fig. 1. Age-related changes in the enzymatic pathways involved in PA/DAG/MAG metabolism in rat cerebellum nuclei. Adult rats (4 mo, white bars) and aged rats (24–28 mo, grey bars) were used to obtain the highly purified nuclear fraction from their cerebellum. All enzymatic assays were performed as previously described [9]. (A) LPPs and DAGL activities, (B) LPAPase activity, (C) PA-PLA activity, (D) DAGL activity and (E) MAGL activity. Statistical analyses were evaluated by Student's *t*-test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$ with respect to the corresponding enzymatic activity values in adult animals.

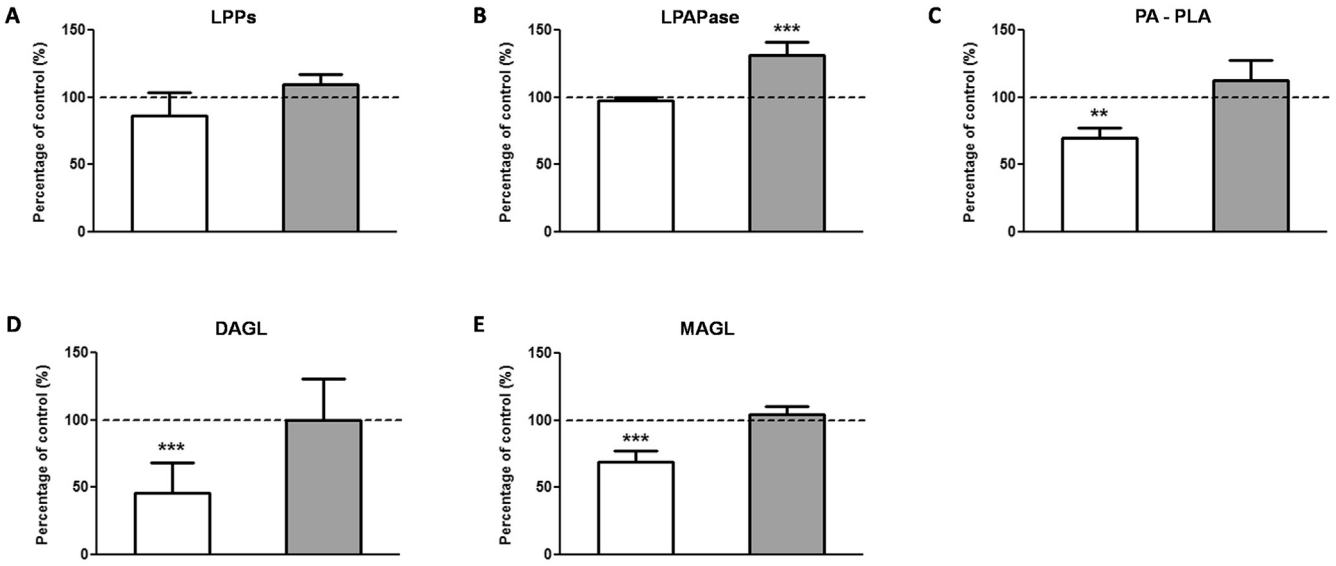

Fig. 2. Effect of RA on PA/DAG/MAG metabolism in adult (white bars) and aged (grey bars) rat cerebellum nuclei. The RA effect was analyzed incubating the highly purified nuclear fraction with RA (10 μM) simultaneously with the individual substrates for each enzyme. (A) LPPs and DAGL activities, (B) LPAPase activity, (C) PA-PLA activity, (D) DAGL activity and (E) MAGL activity. Statistical analyses were evaluated by Student's *t*-test. $^{**}p < 0.005$, $^{***}p < 0.001$ with respect to control (considered as 100% of enzymatic activity). Control represents the enzymatic activity in the presence of RA vehicle (ethanol 0.1%).

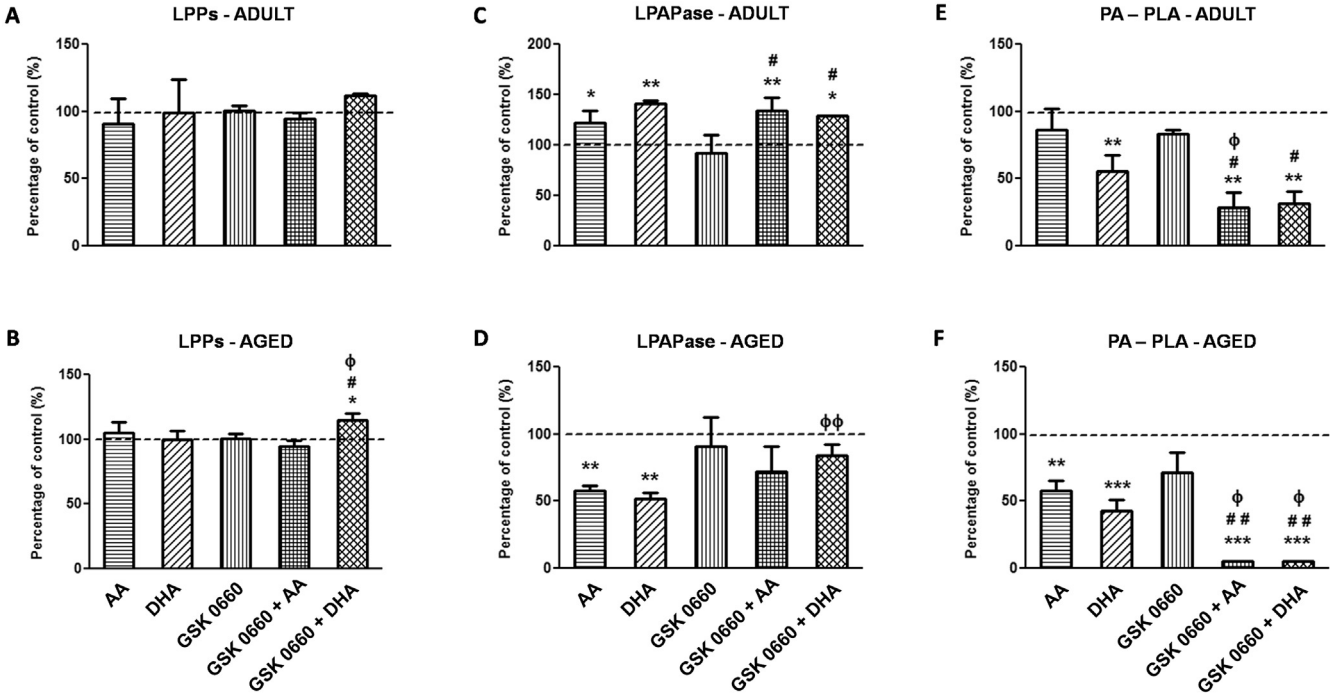

Fig. 3. Effects of AA and DHA on LPPs, LPAPase and PA-PLA activities in adult and aged rat cerebellum nuclei. Isolated nuclei were incubated with 10 μM of AA or DHA simultaneously with the individual substrates for each enzyme. Pre-incubation (10 min) with the PPAR antagonist GSK 0660 (10 μM) was also performed in each enzymatic assay before AA or DHA (10 μM) and substrate addition. Statistical analyses were evaluated by analysis of variance (ANOVA) with Tukey's post-test. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.001$ with respect to control (considered as 100% of the enzymatic activity). Control represents the enzymatic activity in the presence of AA and DHA vehicle (ethanol 0.1%). #p < 0.05, # #p < 0.005 with respect to GSK 0660 condition. Φ p < 0.05 with respect to AA or DHA condition.

in the neurodegenerative processes of aging [16,29]. DAGL and MAGL are the main enzymes responsible for maintaining the levels of 2-arachidonoyl glycerol (2-AG) in the brain [30,31]. PLCβ and DAGLα, both involved in 2-AG production, were, in fact, observed to be expressed in the cerebellum [32] and to co-localize in nuclei from adult rat brain cortical neurons [33]. In recent years 2-AG was found to be involved in various neuroprotective mechanisms [34]. As a consequence, the maintenance of adequate levels of neuroprotective molecules could partially compensate the deleterious effects of aging. In the present study, the activity of DAGL, which is the main enzyme for 2-AG generation, was observed to decrease in aged animals, thus possibly resulting in reduced 2-AG availability.

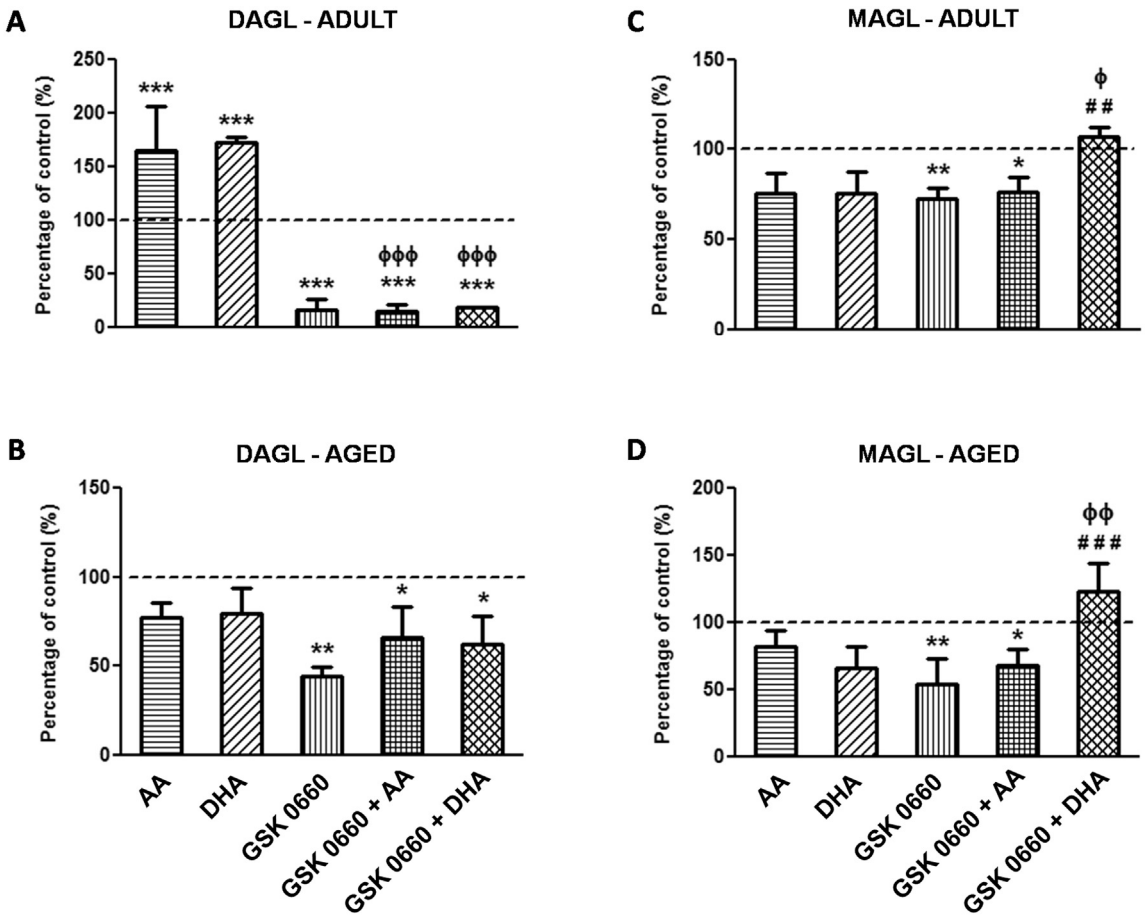

Fig. 4. Effects of AA and DHA on DAGL and MAGL activities in adult and aged rat cerebellum nuclei. Isolated nuclei were incubated with 10 μM of AA or DHA simultaneously with the individual substrates for each enzyme. Pre-incubation (10 min) with PPAR antagonist GSK 0660 (10 μM) was also performed in each enzymatic assay before AA or DHA (10 μM) and substrate addition. Statistical analyses were evaluated by analysis of variance (ANOVA) with Tukey's post-test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$ with respect to control (considered as 100% of the enzymatic activity). Control represents the enzymatic activity in the presence of AA and DHA vehicle (ethanol 0.1%). # #$p < 0.005$, # # #$p < 0.001$ with respect to GSK 0660 condition. Φ $p < 0.05$, Φ Φ $p < 0.005$, Φ Φ Φ $p < 0.001$ with respect to AA or DHA condition.

This could be compensated either by an increase in the activity of LPAPase, another enzyme that generates 2-AG from a 2-arachidonoyl-LPA, or by a decrease in the activity of MAGL, an enzyme that hydrolyzes it. These aging-related changes may thus preserve neuroprotective signaling in nuclei where a nuclear endocannabinoid system could be involved.

RA, DHA and AA have multiple effects not only on cell growth, CNS development and neural plasticity, but also on the modulation of inflammatory processes by means of the regulation of gene expression through RAR or PPAR activation [14,35]. In this respect, it has been demonstrated that RA and some fatty acids have non-genomic effects on cell morphology and on some kinase and transcription factor activities [19,36,37]. It has also been demonstrated that RA modulates different enzymes related to phospholipid metabolism, such as PLD, PLC and PLA_2 in isolated nuclei from LA-N1 cells. In addition, RA which induces differentiation in these cells, was reported to increase nuclear DAG formation from PC, thus suggesting that RA affects PC-PLC or PLD/LPPs activities in this organelle [38–40]. Interestingly, our results show that RA appears to modulate lipid metabolism in isolated nuclei from adult and aged rat cerebellum. Our findings on the effect of RA on nuclear enzymatic activities in adults reveal a RA-dependent non-genomic inhibitory effect on DAGL, MAGL and PA-PLA activities, which may preserve nuclear DAG, MAG and PA levels. Interestingly, these RA-dependent changes have an effect similar to that observed in aging. In both cases, PA-PLA, DAGL and MAGL activities were found to be inhibited whereas only in aged animals was augmented LPAPase activity observed (Fig. 5).

As previously discussed, 2-AG and RA exert their neuroprotective properties through different mechanisms [16,34,41,42]. Based on our observations in nuclei from adult animals, although RA inhibits the DAGL enzyme involved in 2-AG production, this lipid could partially contribute to neuroprotection by diminishing the MAGL activity which prevents 2-AG degradation. The fact that MAG production was observed to be higher only as a result of LPAPase activity strongly suggests in our study that RA differently affects PA metabolism in aged nuclei. This, in turn, leads to the conclusion that in the aging process RA is also involved in neuroprotection by increasing one of the synthesis pathways of 2-AG.

To further clarify the possibility that this metabolism is involved in neuroprotective mechanisms, assays were carried out using other neuromodulators, such as AA and DHA, in adult and aged nuclei. These PUFA are ligands of PPARs. Moreover, other possible ligands of PPARs, such as stearic acid, oleic acid and linoleic acid [43], were tested but produced no changes in the enzymatic activities assayed in our study (data not shown). PPARs are nuclear receptors that heterodymerize with RXR and participate not only in energy metabolism but also in neurodegeneration-related inflammation [16]. Thus, experiments were also performed using GSK 0660, a selective PPARs antagonist, in order to determine possible

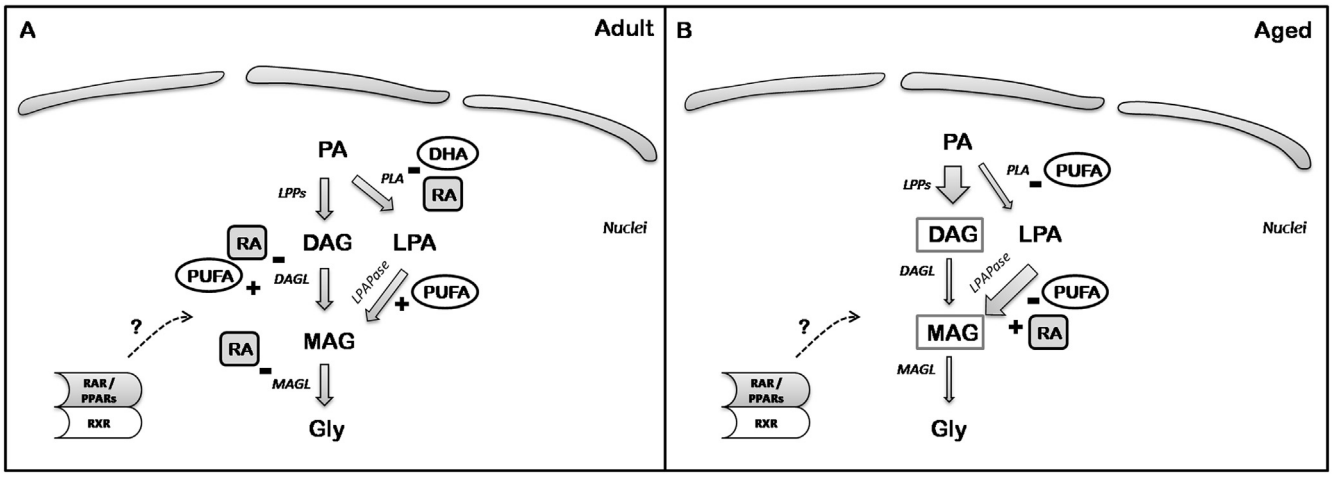

Fig. 5. Scheme showing the principal findings reported in this study on the enzymatic activities involved in lipid metabolism in isolated nuclei from rat cerebellum. (A) Effect of RA, AA and DHA in these enzymes in adult rat cerebellum nuclei. RA inhibits DAGL, MAGL and PA-PLA activities, thus probably leading to higher DAG, MAG and PA availability. In contrast, AA and DHA (PUFA) stimulate both DAGL and LPAPase activities that generate high MAG availability. PA-PLA activity is inhibited by DHA. These effects seem to be PPAR-independent. (B) Age-related changes in LPPs, DAGL, MAGL, PA-PLA and LPAPase activities. Arrow thickness indicates the relative changes in the enzymatic activities. DAG and MAG within squares indicate their high availability as a result of aging. RA stimulates only LPAPase activity which promotes a higher MAG production from LPA. In contrast, PUFA diminishes MAG production due to an inhibition in PA-PLA and LPAPase activities. The PUFA effect on LPAPase activity appears to be PPAR-dependent. The other non-genomic effects of RA, AA and DHA observed both in (A) and (B) could be mediated by a receptor other than PPAR (such as RAR, RXR) or by another unknown mechanism.

PPAR involvement in AA and DHA action. Although AA and DHA do not alter the activities of LPPs in adult and aged animals, a slightly DHA-increasing effect was observed when PPARs were antagonized by GSK 0660 in aged animals, thus indicating that another mechanism, independent of PPARs action, is involved in the DHA effect. The fact that LPAPase activity was observed to increase in adult rats but to decrease in aged rats confirms that AA and DHA generate opposite effects on this enzymatic activity.

In adult rats, the fact that fatty acid effects were observed to remain unmodified even when PPARs were blocked confirmed that their effect on LPAPase activity is PPAR-independent. Conversely, in the presence of GSK 0660 in aged animals, there was no inhibitory effect of AA and DHA on LPAPase, thus indicating that these receptors are involved in this inhibitory effect. On the other hand, the inhibitory effect exerted by DHA on PA-PLA from adult and aged rats and the inhibitory effect exerted by AA on PA-PLA from aged rats was more pronounced in the presence of PPARs antagonist, whereas the effect of AA was evidenced only in the presence of the antagonist in adults. These observations suggest the possibility that the effect of AA and DHA on PA-PLA is PPAR-independent.

DAGL activity was also significantly modified by AA and DHA only in adults. Interestingly, the antagonist alone produced a decrease in DAGL activity both in adult and aged animals. This effect could be related to its ability to exert inverse agonist action on PPARs [44]. Nonetheless, the possible activation of PPARs by an endogenous ligand that is blocked by the antagonist could not be discarded. A direct inhibitory effect of GSK0660 on the enzyme could also occur. The regulation of DAGL activity by AA and DHA in adult animals appeared to be PPAR-dependent, as the subsequent addition of fatty acids after pre-incubation with the antagonist produced no changes in enzymatic activity. Neither in adult nor in aged animals was MAGL activity affected by PUFA. As was observed for DAGL, MAGL activity was also diminished by the presence of the antagonist. Interestingly, the addition of DHA reversed the GSK 0660 effect, reaching control levels in adult and aged nuclei. Thus, DHA modulation in MAGL activity seems to be PPAR-independent. In line with this, previous research has concluded that DHA and AA not only bind to but also activate RXR nuclear receptors. DHA or AA concentrations required for PPAR or RXR activation are, in fact, similar [45]. Thus, AA and DHA effects on PPAR-independent enzymatic activities could also be exerted by RXR activation, as suggested by PPAR blockage, which in turn increases AA and DHA availability for RXR activation. A mechanism other than receptor activation could also be involved. It is worthy of note that both fatty acids yield similar effects on these lipid-related enzymes assayed in adult as well as in aged animals although, unlike DHA, AA is more related to pro-inflammation. Nevertheless, the metabolites produced from DHA and AA are, in fact, those having antagonistic properties with respect to inflammation [46].

5. Conclusion

Summing up, this study shows that LPP, LPAPase, PA-PLA, DAGL and MAGL activities involved in the regulation of lipid signals and messenger levels in nuclei undergo changes in aged animals and are non-genomically modulated by important molecules responsible for the maintenance of neural tissue homeostasis, such as RA and DHA (Fig. 5). As shown by our results, RA as well as AA and DHA produce different effects in aged animals. This indicates a possible role of these nuclear enzymes and their modulators, AA and DHA, in the process of aging. Although further studies are necessary to fully understand the mechanisms involved in this different regulation, our results strongly suggest that lipids at the nuclear level play key roles in pathophysiological neurodegenerative processes.

Acknowledgments

This work was supported by the *Consejo Nacional de Investigaciones Científicas y Técnicas* (CONICET) 112-201101-00437, the *Secretaría General de Ciencia y Tecnología*, *Universidad Nacional del Sur* (UNS) 24/B176, Argentina, the *Fundación Florencio Fiorini* and the *Agencia Nacional de Promoción Científica y Tecnológica* 2014-1089.

Authors are grateful to Translator Viviana Soler for her technical assistance in controlling the use of the English language.

V.L. Gaveglio and N.M. Giusto are research members of CONICET. A.C. Pascual is a post-doctoral fellow of CONICET. S.J. Pasquaré is a research member of UNS.

References

[1] A.N. Hunt, Biochim. Biophys. Acta 1761 (2006) 577–587.
[2] R.W. Ledeen, G. Wu, J. Lipid Res. 45 (2004) 1–8.
[3] K. Tamiya-Koizumi, J. Biochem. 132 (2002) 13–22.
[4] R.G. Cutler, J. Kelly, K. Storie, W.A. Pedersen, A. Tammara, K. Hatanpaa, J.C. Troncoso, M.P. Mattson, Proc. Natl. Acad. Sci. U. S. A 101 (2004) 2070–2075.
[5] A.A. Farooqui, L.A. Horrocks, T. Farooqui, Chem. Phys. Lipids 106 (2000) 1–29.
[6] N.M. Giusto, G.A. Salvador, P.I. Castagnet, S.J. Pasquare, M.G. Ilincheta de Boschero, Neurochem. Res. 27 (2002) 1513–1523.
[7] S.J. Pasquare, V.L. Gaveglio, N.M. Giusto, Arch. Biochem. Biophys. 488 (2009) 121–129.
[8] S.J. Pasquare, G.A. Salvador, N.M. Giusto, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 144 (2006) 311–318.
[9] V.L. Gaveglio, S.J. Pasquare, N.M. Giusto, Arch. Biochem. Biophys. 507 (2011) 271–280.
[10] V.L. Gaveglio, S.J. Pasquare, N.M. Giusto, FEBS Lett. 587 (2013) 950–956.
[11] R. Paul, S.M. Grieve, B. Chaudary, N. Gordon, J. Lawrence, N. Cooper, C.R. Clark, M. Kukla, R. Mulligan, E. Gordon, Neurobiol. Aging 30 (2009) 457–465.
[12] M. Sjobeck, E. Englund, Dement. Geriatr. Cogn. Disord. 12 (2001) 211–218.
[13] P. Antony, L. Freysz, L.A. Horrocks, A.A. Farooqui, Brain Res. Mol. Brain Res. 115 (2003) 187–195.
[14] M. Maden, Nat. Rev. Neurosci. 8 (2007) 755–765.
[15] A.J. Lerner, K. Gustaw-Rothenberg, S. Smyth, G. Casadesus, Biofactors 38 (2012) 84–89.
[16] N.S. van, E. Kampmann, J. Mey, Prog. Neurobiol. 85 (2008) 433–451.
[17] M. Tafti, N.B. Ghyselinck, Arch. Neurol. 64 (2007) 1706–1711.
[18] S. Yu, L. Levi, R. Siegel, N. Noy, J. Biol. Chem. 287 (2012) 42195–42205.
[19] O.S. Gardner, B.J. Dewar, L.M. Graves, Mol. Pharmacol. 68 (2005) 933–941.
[20] N.G. Bazan, J. Lipid Res. 50 Suppl (2009) S400–S405.
[21] V. Casanas-Sanchez, J.A. Perez, N. Fabelo, D. Quinto-Alemany, M.L. Diaz, Front. Physiol. 6 (2015) 203.
[22] S.J. Pasquare de Garcia, N.M. Giusto, Biochim. Biophys. Acta 875 (1986) 195–202.
[23] J. Folch, M. Lees, G.H. Sloane Stanley, J. Biol. Chem. 226 (1957) 497–509.
[24] N.M. Giusto, N.G. Bazan, Exp. Eye Res. 29 (1979) 155–168.
[25] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265–275.
[26] G. Rouser, S. Fkeischer, A. Yamamoto, Lipids 5 (1970) 494–496.
[27] T. Farooqui, A.A. Farooqui, Mech. Ageing Dev. 130 (2009) 203–215.
[28] N.S. Kavok, O.A. Krasilnikova, N.A. Babenko, Exp. Gerontol. 38 (2003) 441–447.
[29] R. Skerrett, T. Malm, G. Landreth, Neurobiol. Dis. 72 (2014) 104–116.
[30] T. Bisogno, F. Howell, G. Williams, A. Minassi, M.G. Cascio, A. Ligresti, I. Matias, A. Schiano-Moriello, P. Paul, E.J. Williams, U. Gangadharan, C. Hobbs, V. Di Marzo, P. Doherty, J. Cell Biol. 163 (2003) 463–468.
[31] T.P. Dinh, D. Carpenter, F.M. Leslie, T.F. Freund, I. Katona, S.L. Sensi, S. Kathuria, D. Piomelli, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 10819–10824.
[32] J. Suarez, F.J. Bermudez-Silva, K. Mackie, C. Ledent, A. Zimmer, B.F. Cravatt, F.R. de Fonseca, J. Comp. Neurol. 509 (2008) 400–421.
[33] C.G. Garcia del, X. Aretxabala, I. Gonzalez-Burguera, M. Montana, J.M. Lopez de, S. Barrondo, R.J. Barrio, C. Sampedro, M.A. Goicolea, J. Salles, J. Neurochem. 132 (2015) 489–503.
[34] A. Bilkei-Gorzo, Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (2012) 3326–3341.
[35] C.I. Janssen, A.J. Kiliaan, Prog. Lipid Res. 53 (2014) 1–17.
[36] J.C. Liou, S.Y. Ho, M.R. Shen, Y.P. Liao, W.T. Chiu, K.H. Kang, J. Cell Sci. 118 (2005) 4721–4730.
[37] S. Masia, S. Alvarez, A.R. de Lera, D. Barettino, Mol. Endocrinol. 21 (2007) 2391–2402.
[38] P. Antony, L. Freysz, L.A. Horrocks, A.A. Farooqui, Neurochem. Res. 26 (2001) 83–88.
[39] P. Antony, J.N. Kanfer, L. Freysz, FEBS Lett. 541 (2003) 93–96.
[40] K. Tamiya-Koizumi, H. Umekawa, S. Yoshida, H. Ishihara, K. Kojima, Biochim. Biophys. Acta 1002 (1989) 182–188.
[41] S.E. O'Sullivan, Br. J. Pharmacol. 152 (2007) 576–582.
[42] M. van der Stelt, V. Di Marzo, Neuromol.. Med. 7 (2005) 37–50.
[43] H.E. Xu, M.H. Lambert, V.G. Montana, D.J. Parks, S.G. Blanchard, P.J. Brown, D.D. Sternbach, J.M. Lehmann, G.B. Wisely, T.M. Willson, S.A. Kliewer, M.V. Milburn, Mol. Cell. 3 (1999) 397–403.
[44] B.G. Shearer, D.J. Steger, J.M. Way, T.B. Stanley, D.C. Lobe, D.A. Grillot, M.A. Iannone, M.A. Lazar, T.M. Willson, A.N. Billin, Mol. Endocrinol. 22 (2008) 523–529.
[45] J. Lengqvist, U.A. Mata De, A.C. Bergman, T.M. Willson, J. Sjovall, T. Perlmann, W.J. Griffiths, Mol. Cell Proteom. 3 (2004) 692–703.
[46] R.P. Bazinet, S. Laye, Nat. Rev. Neurosci. 15 (2014) 771–785.