Age-related expression of adenosine receptors in brain from the senescence-accelerated mouse

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Abstract

Senescence-accelerated mice (SAM) are used as a model of aging and age-associated diseases. SAMP8 are prone strains that show shortened life span and deficits in learning and memory processes, while SAMR1 are strains of accelerated senescence-resistant, long-lived mice. Due to their abnormal APP (amyloid precursor protein) metabolism in brain, SAMP8 may be an Alzheimer-type model. Adenosine receptors are G-protein coupled receptors which are altered in brain from Alzheimer disease (AD) cases. The analysis of adenosine receptors in brain from young (21 days old) and middle-aged (180 days old) SAMP8 as compared with SAMR1 mice revealed differences between these strains associated with age. The age-related increase in mRNA coding A1 and A2A receptors observed in SAMR1 was absent in SAMP8. A1 receptors were significantly decreased with age in SAMR1, while no differences were observed in SAMP8. However, the levels of A1 receptors in young SAMP8 were even lower than those obtained in middle-aged SAMR1. In addition, A2A receptors were significantly increased only in aged SAMR1. A similar age-related decrease in A1 receptors level was also observed in brain from male Wistar rats. These results suggest different age-related effects on adenosine receptors in SAMR1 and SAMP8 strains. Since A1 receptors are mainly neuroprotective, their important loss in very young SAMP8 strain suggests the involvement of these receptors in the pathogenesis of neurodegenerative diseases associated with aging.

1. Introduction

Senescence-accelerated prone mouse strains (SAMP) and resistant strains (SAMR) were established by Takeda and colleagues at Kyoto University by selective breeding from their AKR/J colony in the early 1970s (Takeda et al., 1981). Prone strains of SAM (SAMP8) show shortened life span and early manifestation of senescence with characteristic pathological phenotypes similar to those often observed in elderly humans. These include senile osteoporosis, osteoarthritis, age-related deficits in learning and memory with/without forebrain atrophy, senile amyloidosis, et al. SAMR1 are strains of accelerated senescence-resistant, long-lived mice. The mean life span in the P series is about 9.7 months, 40% shorter than that of the R series (16.3 months). Senescence-accelerated mouse prone strain SAMP8 is one of the most appropriate models to study aging and age-associated diseases because it shows an age-related deterioration of learning and memory at an earlier age, compared with the control mouse R1 strain in senescence-accelerated resistant mouse (SAMR1) (Miyamoto et al., 1986; Miyamoto, 1997; Nomura and Okuma, 1999; Butterfield and Poon, 2005). Alteration of learning and memory of SAMP8 have been associated with a decline in neurotransmitter activity as well as decreased serotonin, increased GABA and decreased cholinergic activity (Morley et al., 2002b). In addition, cognitive defect in these animals can be due to overproduction of β-amyloid peptide (AβP) being reversed by specific antibodies to β-amyloid. Furthermore, SAMP8 mice present lower mRNA levels of apolipoprotein E and increased levels of presenilin-2 when compared to SAMR1 (Wei et al., 1999). Therefore, these animals have been suggested as a good model for the study of Alzheimer’s disease (Morley, 2002; Morley et al., 2002a; Banks et al., 2007; Pallàs et al., 2008).

SAMP8 mice also present other characteristics that contribute to their pathological phenotype. SAMP8 mice present a lower expression of neurotrophic genes as glial cell derived neurotrophic factor (GDNF) (Miyazaki at al., 2003), neurotrophin-3 (NT-3) and nerve growth factor (NGF) (Kaisho et al., 1994). Furthermore, the expression of proteins involved in reactive oxygen species (ROS)
metabolism is altered in SAMP8 mice, which may be involved in the increased oxidative stress found in SAMP8 mouse brain. Compared to SAMR1 mice, the activity of enzymes involved in antioxidanid processes are decreased in the cerebral cortex of aged SAMP8. This has been reported for manganese superoxide dismutase (Mn-SOD), glutamine synthase (GS), glutathione peroxidase (GPx) and peroxidase activities (Kurokawa et al., 2001; Sato et al., 1996; Okatani et al., 2002). Moreover, other enzymes which contribute to ROS generation, such as nitric oxide synthase (NOS), are increased in aged SAMP8 mice (Inada et al., 1996).

Adenosine is a nucleoside widely distributed in central and peripheral nervous system that exerts its actions through four types of receptors named A1, A2A, A2B and A3, all of them being peripheral nervous system that exerts its actions through four G-protein coupled receptors (GPCR). A1 and A3 receptors are coupled to stimulation of the enzymatic activity, through Gi/o proteins, to adenylyl cyclase activity inhibition, while A2A and A2B receptors are coupled to stimulation of the enzymatic activity, through Gs protein (Ralevic and Burnstock, 1998; Fredholm et al., 2001, 2005). Out of the four adenosine receptors, the A1 subtype is the most abundant and widespread in the brain, where it plays a neuroprotective role because of its capacity to decrease the release of excitatory neurotransmitters, mainly glutamate (Dunwiddie and Masino, 2001). A2A receptors are concentrated in the basal ganglia but they are also present throughout the brain, albeit in a considerably lower density. A2B and A3 receptors are the least abundant in the brain (Cunha, 2005).

Changes in adenosine receptors during aging have been demonstrated in several animals (Cunha, 2005) and in humans (Meyer et al., 2007). Decreased expression and density of A1 in the cortical and hippocampal regions has been observed (Cheng et al., 2000; Cunha et al., 1995, 2001a,b; Pagonopoulou and Angelatou, 1992) whereas the density of A2A receptors increases with age (Cunha et al., 1995; Cunha, 2005; Lopes et al., 1999). However, these age-related changes in adenosine receptor levels observed in some brain areas have not been detected in the striatum, where these levels might be only slightly affected by aging (Cunha et al., 1995). Beyond this, adenosine receptors have not been analyzed in SAM strains until now. The SAMP8 mouse has been proposed as an excellent model for studying the pathogenesis of learning and memory disturbances associated with Aβ overproduction. The identification of senile plaques and the increased expression of Aβ in this mouse model suggest it may be an acceptable model for Alzheimer’s disease (Morley, 2002; Morley et al., 2002a,b; Banks et al., 2007; Pallas et al., 2008). We have previously reported that adenosine A1 and A2A receptors are altered in frontal cortex brain from AD patients (Albasanz et al., 2008). The aim of the present work was to determine the expression of different adenosine receptors, mainly A1 and A2A, and their possible age-related changes in brains from SAMR1 and SAMP8 strains, in order to use these animals as models to study the mechanism involved in the neuropathogenesis of Alzheimer’s disease.

2. Materials and methods

2.1. Materials

Cyclopentyl-1,3-dipropylxanthine,8-[diprop-2,3-3H(N)] ([3H] DPCPX) 120 Ci/mmol and adenosine 3’-cyclic phosphate [2,8-3H] ([3H]cAMP) 27.4 Ci/mmol were purchased from PerkinElmer (Madrid, Spain). Anti-A1 antibody was purchased from Calbiochem (Bionova, Madrid, Spain), anti-A2A antibody from Upstate (Millipore, Madrid, Spain) and anti-β-actin from Abcam (Cambridge, UK). Guanosine-5’-O-(3-thiotriphosphate) tetralithium salt (GTP[S]) and calf intestine adenosine deaminase (ADA) were purchased from Roche (Barcelona, Spain). Creatine kinase, creatine phosphate, protein kinase A, 4-[3-butoxy-4-methoxybenzyl]imidazolidin-2-one (Ro 20-1724), forskolin, N6-cyclopentyladenosine (CPA), N6-cyclohexyladenosine (CHA), adenosine 5’-triphosphate (ATP) and guanosine 5’-[β,γ-imido]triphosphate (Gpp(NH)p) were from Sigma (Madrid, Spain). All other reagents were of analytical grade and obtained from commercial sources.

2.2. Animals

Young (21 days old) and middle-aged (180 days old) SAMR1 and SAMP8 mice or young male (3 months) and aged male (24 months) Wistar rats were kept at constant temperature (25 ± 1 °C) and humidity, maintained on a 12 h light/12 h dark cycle with ad libitum access to rodent food and water. All experiments followed the European Union regulations about animal experimentation and those of the Experimentation Animal Committee of the Castilla-La Mancha University. Special care was taken to minimize animal suffering and to reduce the number of animals used.

2.3. Plasma membrane isolation

Plasma membranes from mouse and rat brain were isolated as described previously (León et al., 2002). Samples were homogenized in 20 volumes of isolation buffer (50 mM Tris–HCl, pH 7.4, containing 10 mM MgCl2 and protease inhibitors) in Dounce homogenizer (10 × A, 10 × B). After homogenization, whole brain preparations (excluding cerebellum and spinal cord) were centrifuged for 5 min at 1000g in a Beckman JA 21 centrifuge. Supernatant was centrifuged for 20 min at 27,000g and the pellet was finally resuspended in isolation buffer.

2.4. [3H]DPCPX binding assays to plasma membranes

Binding assays to plasma membranes from mouse and rat brain were performed as described previously (León et al., 2002). Plasma membranes were incubated with 5 μM adenosine deaminase (ADA) in 50 mM Tris, 2 mM MgCl2, pH 7.4, for 30 min at 25 °C in order to eliminate endogenous adenosine from membrane preparations. Then, plasma membranes (50–75 μg of protein) were incubated with [3H]DPCPX for 2 h at 25 °C. Saturation assays were carried out at different [3H]DPCPX concentrations (0.1–20 nM) using CPA at a concentration 10–3 times higher than the radioligand, in order to obtain non-specific binding. Binding assays were stopped by rapid filtration through Whatman GF/B filters, previously pre-incubated with 0.3% polyethyleneimine using a FilterMate Harvester (Perkin–Elmer). Scintillation liquid mixture was added in order to measure radioactivity in a Microbeta Trilux (Perkin Elmer) liquid scintillation counter.

When the effect of Gpp(NH)p was studied [3H]DPCPX binding assays were performed at saturation concentration using the same methodology with minor modifications. Briefly, the buffer employed was 50 mM Tris, pH 7.4, containing 10 μM Gpp(NH)p, and the final concentration of [3H]DPCPX used was 20 nM.

2.5. Western blot assays

For Western blot assays, 50 μg of protein was mixed with loading buffer containing 0.125 M Tris (pH 6.8), 20% glycerol, 10% β-mercaptoethanol, 4% SDS and 0.002% bromophenol blue, and heated at 95 °C for 5 min. Protein was electrophoresed on a 10% SDS–PAGE gel using a mini-protein system (Bio-Rad) with molecular weight standards (Bio-Rad). Protein transfer to nitrocellulose membranes was carried out in iBlot® Dry Blotting System (Invitrogen). Membranes were washed with PBS–Twee 20, blocked with PBS containing 5% skimmed milk, and then incubated with the primary antibodies at 4 °C overnight (1:1000 dilution for anti-A1R,
1:500 dilution for anti-A2AR and 1:5000 dilution for anti-β-actin). After rinsing, the membranes were incubated with the corresponding secondary antibody (Bio-Rad) at a dilution of 1:5000 in PBS containing 5% skimmed milk for 30 min. Antigen was visualized using the ECL chemiluminescence detection kit (Amersham), and specific bands were quantified by densitometry using Multianalyist 1.0 software (Bio-Rad). Membranes were stripped for a second probe by incubating with 0.15% glycine, 0.1% SDS, and 1% Tween 20, pH 2.2, for 30 min at room temperature. After removing the stripping buffer and washing them twice with PBS, membranes were ready for the blocking stage.

2.6. Preparation of total RNA and cDNA

Total RNA was extracted from whole brain (excluding cerebellum and spinal cord) using an ABI 6100 Nucleic Acid PrepStation according to the manufacturer’s protocol. All chemicals for the ABI 6100 were purchased from Applied Biosystems (Foster City, CA). Total RNA from mice was isolated and stored individually at −80 °C. Ratio of A2AR/A2B (purity of RNA) was in the range 1.9–2.1. RNA concentrations were determined using Applied Biosystems High-Capacity cDNA Archive Kit.

2.7. Quantitative real time RT-PCR analysis

Quantitative real time RT-PCR analysis (Higuchi et al., 1993) was performed with an Applied Biosystems Prism 7500 Fast Sequence Detection System using TaqMan® universal PCR master mix according to the manufacturer’s specifications (Applied Biosystems Inc., Foster City, CA). The validated TaqMan probes and primers for A1 (assay ID: Rn00567668_m1), A2A (assay ID: Rn00583935_m1), A2B (assay ID: Rn00567697_m1), A3 (assay ID: Rn00563680_m1) and β-actin (assay ID: Rn00667869_m1) were assay-on-demand gene expression products (Applied Biosystems). The TaqMan® primer and probe sequences are packaged together in a 20× solution. Rat β-actin gene was used as endogenous control. The thermal cycler conditions were as follows: hold for 20 s at 95 °C, followed by two-step PCR for 40 cycles of 95 °C for 3 s, followed by 60 °C for 30 s. Levels of RNA expression were determined using the GraphPad Prism 5.0 program for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Expression of genes coding adenosine receptor types in SAMR1 and SAMP8 mice

To determine the age-related changes in expression of gene coding adenosine receptors in SAMP8 and SAMR1 mice, we isolated total RNA from both strains and performed quantitative real time PCR using selective oligonucleotides to adenosine A1, A2A, A2B and A3 receptors. The ages of animals were 3 weeks (21 d) and 6 months (180 d). Results show that mRNA coding adenosine A1 receptors are significantly increased in middle-aged versus young SAMR1 animals, suggesting an age-associated up-regulation in resistant control mice. However, the level of A3 mRNA in young and middle-aged SAMP8 animals was not significantly different – of the same order as that obtained in young SAMR1 (Fig. 1A). Similar results were obtained for A2B receptors whose level was increased in middle-aged versus young resistant mice but was not altered in SAMP8 (Fig. 1D). Concerning A2A receptors, no significant differences were obtained between young and middle-aged animals, either in SAMP8 or in SAMR1. However, the level of A2A mRNA expression was significant lower in SAMP8 versus SAMR1 (Fig. 1C).

3.2. Adenosine A1 receptor in SAMR1 and SAMP8 mice

Adenosine A1 receptors are widely distributed in the brain and they are involved in neuroprotection. We measured adenosine A1 receptors in plasma membranes from SAMR1 and SAMP8 mice by binding assay using [3H]DPCPX, a selective A1 receptor antagonist, as radioligand. In all cases assayed saturation curves were better fitted to one binding site model. Results from saturation curves showed a significant decrease in total adenosine A1 receptor numbers in middle-aged SAMR1 (38%), suggesting a loss of receptors associated with aging (Fig. 2A and C). This decrease was not observed in the SAMP8 strain (Fig. 2B and C). However, the total adenosine A1 receptor number in SAMP8 was significantly lower than in SAMR1, with even fewer receptors detected than in middle-aged SAMR1 mice, suggesting that A1 receptors are already
altered at a very early age in SAMP8. No significant age-associated differences in Kd values were observed in any SAM strain. However, Kd values in SAMP8 strain were lower than in SAMR1, suggesting a higher receptor affinity in the former (Fig. 2D).

Due to the discrepancy observed in data obtained by real time PCR and by radioligand binding assays in these samples, additional binding assays were carried out in the presence of Gpp(NH)p, a non-hydrolizable GTP analogue, to rule out possible [3H]DPCPX activity as an inverse agonist (Finlayson et al., 2003). Results obtained (Fig. 3) showed a significant decrease in [3H]DPCPX binding in middle-aged SAMR1, young SAMP8 and middle-aged SAMP8 as compared to [3H]DPCPX binding levels in young SAMR1 strain. These results correlate with binding data obtained in the absence of Gpp(NH)p and confirm [3H]DPCPX as a selective antagonist.

To measure adenosine A2A receptors, Western blot experiments were performed with membranes from SAM animals. Results showed a significant age-related increase in the level of A2A receptors in SAMR1, while no significant difference was observed in SAMP8 related to age. Moreover, levels of A2A receptors observed in both young and middle-aged SAMP8 were of the same order as those obtained from young SAMR1 (Fig. 4). In addition, to confirm the variation of A1 receptors detected by radioligand binding assay, Western blot experiments were also performed using anti-A1 receptor antibody. Results show an aged-associated loss of adenosine A1 receptors in SAMR1 mice (Fig. 5). In addition, the level of A1 receptors in very young SAMP8 mice was of the same order as that observed in middle-aged SAMR1 animals, confirming results obtained by saturation binding assays.

3.3. Adenosine A1 receptors/AC activity in Wistar rats

To compare and confirm the effect of aging on adenosine A1 receptor levels we also analyzed the status of these receptors in brain from Wistar rats. Two groups of animals were used, young (3 months) and old (24 months) male rats. Saturation curves obtained by radioligand binding assays showed a significant decrease in adenosine A1 receptors in brain from old versus young rats (49.3% of young rats) without significant variation in receptor affinity (Fig. 6).

To evaluate whether age-related variation in adenosine receptors from Wistar rats modulates the transduction pathway mediated by these receptors, we analyzed the status of adenyl cyclase activity in young and old rats. Results show that basal and forskolin or forskolin plus GTPγS-stimulated adenyl cyclase activity were significantly lower in old than in young animals (Fig. 7A). Moreover, the ability of CHA, a selective A1 receptor agonist, to inhibit this enzymatic activity, was also decreased, suggesting a desensitization of the response system associated with the loss of receptors in the plasma membrane (Fig. 7B).
4. Discussion

To our knowledge, this study is the first to deal with the expression of mRNA coding different adenosine receptors and to quantify adenosine A1 and A2A receptors in brain from SAMR1 and SAMP8 mice. Results presented herein show an age-related loss of adenosine A1 receptors in SAMR1, associated with an increase in the rate of synthesis of this receptor, probably as a compensatory mechanism to prevent the important loss of receptor detected at the membrane surface. In contrast, an age-related increase in adenosine A2A receptors was observed in SAMR1 with no variation in SAMP8. However, the most important finding in our study is that adenosine A1 receptors, which have been described as being neuroprotective, are already severely decreased in very young SAMP8 mice (3 weeks old) suggesting a great alteration of these receptors in this aging model.

Morphological changes in the aging brain have been described and they depend on the species, the strains and the ages included in the study, as well as on individual differences. Changes in A1 and A2A receptors and their function during aging have been demonstrated, with A1 being decreased and A2A increased, respectively (Cunha, 2005; Rodrigues et al., 2008). In spite of the lack of anatomical resolution in our study, our findings of age-related changes in A1 receptors in normal aging mice (SAMR1) are consistent with previously published data. Age-related changes in adenosine A1 receptor binding have been described in mouse brain using the selective agonist CHA. In the cortex, hippocampus, and cerebellum of aged mice (28 months old), a significant decrease of about 44%, 50% and 12%, respectively, was observed in CHA binding compared to young animals (3 months old), without changes in Kd values and not dependant on a general cell degeneration in old age (Pagonopoulou and Angelatou, 1992). Also in mice, there has been confirmed to be widespread age-related loss of A1 receptors in various cortical and subcortical structures involved in the neuromodulatory role of adenosine (Ekonomou et al., 2000).

The methodology employed to quantify A1 receptors in SAM mice was validated by our results in male Wistar rats, which agree with those published by Cunha and co-workers in which the density of A1 binding sites in aged rats (24 months) was reduced by 42% in whole brain membranes (Cunha et al., 2001b) by 33% in the hippocampus, and by 60% in the cortex, when compared with young adult rats (6 weeks), with no changes in Kd (Cunha et al., 1995). A significant decrease in DPCPX binding to hippocampal membranes of aged versus young rats has also been observed (Sperlágh et al., 1997). By immunoblotting analysis there was shown to be an age-related decrease in A1 receptor in cerebral cortex from Wistar rats. Compared to preparations from 2-month-old animals, the levels of A1 in 6, 12 and 24-month-old rats were re-
Reduced by 14%, 32% and 28%, respectively (Cheng et al., 2000). In addition, the pattern of binding of the A1 receptor antagonist [3H]DPCPX decreased with aging in membranes from whole brain of male Wistar rats while Kd values were not different among age groups (Cunha et al., 2001b).

Loss of A1 receptors in plasma membrane has been associated with a down-regulation of adenosine receptor by ligand overexposure. Down-regulation and desensitization of adenosine A1 receptors in vivo has been suggested as being induced by high adenosine levels (León et al., 2002; León et al., 2004, 2005a,b) and it comprises the classical GPCR response when exposed chronically to agonist (Hanyaloglu and von Zastrow, 2008). In agreement with this hypothesis, we observed a decrease in A1 receptor binding in plasma membranes from SAMR1 and SAMP8 mice. The decrease in binding was significant in the absence of Gpp(NH)p, while in the presence of Gpp(NH)p, there were no statistically significant differences between young and old animals.

Fig. 3. Gpp(NH)p effect on radioligand binding assay in SAM mice. [3H]DPCPX binding assays were performed in plasma membranes (50–75 μg of protein) from SAMR1 or SAMP8 mice. After pre-incubation with adenosine deaminase, binding assays were performed with 20 nM [3H]DPCPX in the presence of 10 μM Gpp(NH)p, without MgCl2, and in the presence of MgCl2, without Gpp(NH)p. CPA was used to obtain non-specific binding. Data are means ± SEM of independent experiments performed in duplicate using different membranes isolated from four animals of each condition. No statistical differences were observed between binding data obtained in the presence or the absence of Gpp(NH)p. *p < 0.05, **p < 0.01 significantly different from control young SAMR1 mice in the absence of Gpp(NH)p; ***p < 0.05 significantly different from control young SAMR1 mice in the presence of Gpp(NH)p.

Fig. 4. Western-blot of A2AR in plasma membranes from SAM mice. Fifty micrograms of protein were subjected to SDS/PAGE, transferred electrophoretically to nitrocellulose and probed with antisera anti-A2A, as described in Section 2. Upper panel shows a representative autoradiography, showing the bands corresponding to A2AR and β-actin, which was used as gel loading control. Lower panel shows the obtained histogram. Data are expressed as means ± SEM using arbitrary units. *p < 0.05 significantly different from control young SAMR1 mice.

Fig. 5. Western-blot of A1R in plasma membranes from SAM mice. Fifty micrograms of protein were subjected to SDS/PAGE, transferred electrophoretically to nitrocellulose and probed with antisera anti-A1, as described in Section 2. Upper panel shows a representative autoradiography, showing the bands corresponding to A1R and β-actin, which was used as gel loading control. Lower panel shows the obtained histogram from densitometric analysis of bands. Data are expressed as means ± SEM using arbitrary units. *p < 0.05 and ***p < 0.001 significantly different from control young SAMR1 mice.

Fig. 6. Adenosine A1 receptor detection in Wistar rats by radioligand binding assay. Saturation curves of [3H]DPCPX binding to plasma membranes were performed by incubation of 50–75 μg of protein from young and old rats with increasing concentrations of the radioligand, as described in Section 2, after pre-incubation with adenosine deaminase in order to remove endogenous adenosine. Total receptor number (Bmax) and receptor affinity (Kd) determined by Scatchard and nonlinear regression analysis are shown in the inset. Data are means ± SEM of number of experiments in brackets performed in triplicate using different membranes from different animals. *p < 0.01 significantly different from young rats.

**p < 0.05 and ***p < 0.001 significantly different from control young SAMR1 mice.

Adenyl cyclase activity in brain plasma membranes from Wistar rats. (A) Ten to 20 micrograms of plasma membranes, previously incubated with adenosine deaminase, were used to determine basal cAMP level and stimulation after incubation with 10 μM forskolin (Fsk) or 10 μM Fsk plus 5 μM GTPγS. (B) Ten to 20 micrograms of plasma membranes were incubated with Fsk plus GTPγS in the presence and in the absence of CHA, a selective A1R agonist. cAMP accumulation was measured as described in Section 2. Data are means ± SEM of at least four experiments performed in triplicate with different membrane preparations from different rats.*
Although no references are available about adenosine content in SAM brain, at least to our knowledge, changes in neurotransmitter levels in SAM animals have been reported as increasing in GABA and decreasing in acetylcholine and serotonin (Morley, 2002). In the hippocampus and/or cerebral cortex of P8 strain at early ages, the contents of glutamate (Glu) and glutamine (Gln) were higher than in R1, while the aspartate (Asp) and alanine (Ala) content was lower, which suggests that a metabolic pathway from alpha-ketoglutarate to Glu predominates in SAM8 brain (Kitamura et al., 1992; Nomura et al., 1991). Local accumulation of excitatory amino acids can lead to neuronal degeneration because of the neurotoxic effects at high concentrations of Glu and analogues in Glu receptive areas (Olney, 1990). Increased glutamate levels have been found in several brain areas in AD and in cerebrospinal fluid (CSF) from AD patients (Procter et al., 1988; Pomara et al., 1992). This excess of endogenous glutamate would contribute to cell death in AD, possibly as the result of the failure to remove glutamate from synapse (Greenamyre and Young, 1989). In agreement, a down-regulation and desensitization of metabotropic glutamate receptors in the frontal cortex in common forms of dementia with a down-regulation and desensitization of metabotropic glutamate receptors in the frontal cortex in common forms of dementia with AD and in Alzheimer disease cases have been detected (Albasanz et al., 2005). The neuroprotective role of adenosine A1 is mainly due to inhibition of glutamate release through adenosine A1 receptor activation. Therefore, the loss of A1 receptors could be responsible for the increase in the glutamate level in SAM mice.

In summary, in SAMR1 mice used here as controls, it was shown that there is an age-related loss of adenosine A1 receptor, as also observed in rats, but at early stages. Moreover, the most important result in this work is that in brain from very young SAM8 mice there is a reduced A1 receptor level similar to that obtained in aged SAMR1, suggesting an important alteration of these receptors at only 3 weeks of life which is maintained thereafter. In vivo PET study detected an age-dependent A1 receptor loss in humans that may be of pathophysiological importance in various neurological diseases associated with aging (Meyer et al., 2007). Therefore, SAM mice may represent an important model in the study of alteration of neurotransmitter receptors related to neurodegenerative diseases.

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