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# Modulation of Signal Transduction Pathways in Senescence-Accelerated Mice P8 Strain: A Useful Tool for Alzheimer's Disease Research

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## 1. Introduction

Senescence-accelerated mouse (SAM) lines serve as models of aging and age-associated diseases. The SAMP8 strain has a shortened life span and early-onset manifestations of senescence with characteristic pathological features observed in elderly humans, including deficits in learning and memory. In brains of SAMP8 mice, the processing of amyloid precursor protein (APP) is altered, resulting in excess production and accumulation of amyloid-β peptide (Aβ), tau is hyper-phosphorylated, and oxidative stress is increased. These phenotypic abnormalities are quite reminiscent of the findings in human brains with Alzheimer's disease (AD). Mechanistically, metabolic pathways that are responsible for the generation of reactive oxygen species (ROS) are increased, while antioxidant systems are reduced in activity in the cerebral cortex of aged SAMP8 mice. Besides these structural and metabolic alterations, brains of aged SAMP8 mice exhibit neurochemical abnormalities such as altered signaling through G protein-coupled receptors for 5-hydroxytryptamine, acetylcholine, adenosine, dopamine, melatonin, glutamate and GABA, ion channel receptors, and nuclear hormone receptors (e.g. for all-trans-retinoic acid, cortisol or estradiol). Consequences include alterations in the levels of neurotransmitters, receptor numbers, receptor binding affinity, and second messengers. Of note is that in AD, G protein-coupled receptors and/or their corresponding signaling pathways are often impaired. Together, the observations in aged SAMP8 mouse brains provide convincing evidence that

this model serves as an excellent research tool for studying AD pathogenesis and strategies for treatment. Additionally, many of the pathological and neurochemical abnormalities in SAMP8 mice are linked to altered expression of genes that are integrally related to processes such as neuroprotection, signal transduction, protein folding/degradation, intracellular transport and immune response. Several studies have already utilized pharmacological or dietary measures to restore cognitive function and enhance neuroprotection in aged SAMP8 mice, suggesting that these approaches may have applications in the treatment of AD. This review compiles available data concerning the signaling pathways that are altered in SAMP8 mice, and compares the effects to known abnormalities in AD brains.

## 2. Senescence-Accelerated Mouse model hallmarks: the interest in SAMP8 strain

The senescence-accelerated mouse (SAM) model of accelerated senescence was originally established by Dr. Takeda at the University of Kyoto through phenotypic selection from a common genetic pool of the AKR/J strain. After continuous sister-brother mating to maintain the line under conventional conditions, some littermates were found to differ from the original offspring. This distinct subset of mice exhibited an unusual phenotype characterized by early-age onset senility and shortened lifespan. Five litters with this new phenotype were selected as progenitors of the senescence-accelerated-prone mice (SAMP), while 3 litters with normal ageing were selected as progenitors of the senescence-accelerated-resistant mice (SAMR) (Takeda et al., 1981). Fourteen SAMP and four SAMR litters were produced from the original breeding pairs (Takeda, 1999). In the SAMP mice, the first signs of premature aging were hair loss, reduced physical activity, ophthalmic disorders, and shortened lifespan (mean lifespan of 9.7 months versus 16.3 months for SAMR mice) (Takeda et al., 1994). These and other characteristic features of SAMP mice are summarized in Table 1.

The use of experimental models to study the beginning and the course of senescence and, in a pharmacological approach, to design drugs capable of delaying this process has been widely accepted. Some examples of these experimental approaches are the development of genetically modified organisms, which overexpress genes related to aging, chemical increase of free radicals, viral inoculation, pharmacological interference with some nerve and endocrine functions, and stress induction, among others. These are very useful approaches but present some disadvantages which are not within the scope of this chapter to describe. Moreover, in recent years there has been brought to light a need for improved animal models as a critical barrier in the study of several neurodegenerative disorders. In the particular case of AD study, a direct consequence of the knowledge of AD etiology is that scientists are not able to design an animal model that fully resembles AD pathology. Furthermore, time is not on our side; it has been estimated that nowadays AD affects at least 30 million people around the world, death occurs within 10 years of diagnosis (Lee & Chodosh, 2009), and predictions are not encouraging at all. In the year 2050 the number of ageing people will be on the order of two billion people (WHO data and statistics), most of them in developed countries, which would exponentially increase the number of Alzheimer's patients. Nowadays, the available animal models of AD are restricted to the overexpression of genes with specific mutations associated with early-onset familial AD, which only represents ≈5% of AD cases— considered by many researchers, although useful,

to be models with poor clinical relevance and limited predictive value. The use of SAM strains has allowed researchers to pursue a more biological approach to study senescence processes. As may be observed in the central column of Table 1, the main feature of all SAM strains is accelerated senescence, which is the reason why they are a widely used animal model to study geriatric disorders. It is necessary to establish the difference between *accelerated* senescence and *pathologic* senescence. The former is the biological phenomenon observed in living organisms as time goes by. In this case, the observer only reports a faster course of events when compared to physiologic senescence (which is also convenient from a technical point of view). The latter refers to a pathological phenomenon that alters aging process by interacting with biological processes, inducing *premature* senescence.

| **Strain** | **Characteristics** | **Geriatric disorders** |
|---|---|---|
| SAMP1 | Senile amyloidosis<br>Impaired immune system<br>Impaired auditory system<br>Retinal atrophy<br>Hypertensive vascular disease<br>Contracted kidney<br>Pulmonary hyperinflation | Presbycusis<br>Atrophy of the retina<br>Senile cataracts<br>Senile lung |
| SAMP2 | Senile amyloidosis<br>Impaired immune system | Senile cataracts<br>Senile lung |
| SAMP3 | Degenerative arthrosis | Degenerative joint disease |
| SAMP6 | Senile osteoporosis | Senile osteoporosis |
| SAMP7 | Senile amyloidosis<br>Thymoma | |
| SAMP8 | Age-related learning and memory deficits<br>Anxiety<br>Impaired immune system<br>Age-dependent deposition of amyloid β-peptide | Deficits in learning and memory<br>Emotional disturbances<br>Abnormal circadian rhythm<br>Damage to the blood-brain barrier |
| SAMP9 | Age-related cataracts | Senile amyloidosis |
| SAMP10 | Brain atrophy<br>Age-related learning and memory deficits<br>Age-related depression | Deficits in learning and memory<br>Forebrain atrophy<br>Abnormal circadian rhythm |
| SAMP11 | Age-relating thickening of tunic media of thoracic aorta<br>Senile amyloidosis<br>Contracted kidney | Diffuse medial thickening of the aorta |

Table 1. Summary of the main features of different strains of SAMP model. From Butterfield & Poon, 2005 and Chiba et al., 2009.

During the past century, a considerable number of theories have been proposed to explain the nature of aging from the different points of view which are involved in it: molecular, cellular, tissue level, etc. This has made it a difficult task to achieve. Nowadays, the scientific

community is still debating which of these theories is the one to explain all the changes reported during aging, but although there is not a universally accepted theory of aging, the free radical theory (Harman, 1956) seems to be the one with the most adherents. This theory postulates that changes observed during aging are due to the direct action of free radicals, generated during cellular metabolism, over biomolecules. Hence, chemical oxidation of proteins, DNA, lipid membranes and other biomolecules induced by reactive oxygen species (ROS) leads to cellular dysfunction and aging in humans and animals. Furthermore, ROS damage has been described as the first pathologic event that occurs in the brain of AD patients (Nunomura et al., 2001) and in SAMP8 strain (Pallas et al., 2008). Following these facts, the SAMP8 strain seems to be a valid animal model for the study of AD as the molecular mechanism that generates age-related impairments (even if it is not the only one, Zhu et al., 2007) in both senescent mouse and AD patients appears to be the same.

SAMP8 strain has been extensively studied by comparing aged SAMP8 mice with aged SAMR1 mice, and also aged SAMP8 mice with young SAMP8 mice. In both cases, neuropathological, neurochemical, and, especially, behavioral/cognitive abnormalities found in aged SAMP8 mice are similar to the deficits observed in AD patients and in some genetically modified animals. Moreover, the chronology of AD symptoms and appearance of pathology are closer to abnormalities described in SAMP8 strain than to what is reported in other genetically modified animals (Pallas et al., 2008). Recently, SAMP8 strain has caught the attention of several investigators, owing to its unique characteristics, with special focus in their use as neurodegenerative models (Pallas et al., 2008; Takeda, 2009). This strain spontaneously develops a pathologic phenotype characterized by age-related disorders, such as learning and memory deficits, mood disorders, such as reduced anxiety-like behavior and depressive behavior, and abnormality of circadian rhythm. In addition, when APP cDNA from SAMP8 strain was sequenced, familial AD mutations were not found (Kumar et al., 2001), suggesting that the age-related disorder reported in this strain was probably not following the same pathways as observed in familial AD. Furthermore, Aβ levels found in SAMP8 mice seem to be closer to those observed in AD patients than what has been reported in genetically modified mice (Rosenberg, 2000). This is, precisely, SAMP8's main characteristic: the age-related phenotype is developed spontaneously and in almost the same order as reported in human beings. That is the reason why this animal model is a high value-added model; neurodegenerative observations reported in this model are not due to the introduction or the deletion of a gene(s) in an animal model but are directly induced by physiological processes.

Age-related deficits in SAMP8 strain have been known for a long time. Since their first description by Dr. Takeda, several laboratories have associated behavioral changes to age-related cognitive impairment. However, Dr. Takeda's laboratory was the first to globally describe age-related cognitive deficits in SAMP8 mice compared to SAMR1 age-matched controls (Miyamoto et al., 1986). They reported an age-associated increase in spontaneous motor activity, impairment in the acquisition of passive avoidance response at 2 months of age, and impairment in the acquisition of active avoidance response at 12 months of age. Alternative paradigms designed to test other behavioral tasks, such as spatial memory acquisition, are particularly useful to study age-related deficits, as memory and learning deficiencies are directly dependent on hippocampal function. These experiments, such as multiple T-maze and Morris water maze, showed an age-related impairment of spatial memory acquisition in 2-month-old SAMP8 mice. Nevertheless, these results turned controversial as other groups found different onset times for SAMP8 age-related

impairments in learning and memory depending on the test used (reviewed in Flood & Morley, 1998). Taken together, all these studies suggest that learning and memory tasks are impaired in SAMP8 mice at early age which will be detected at one or another age depending on the specific aspect of learning and memory measured by the experimental test used. These age-related cognitive deficits found at an early age in SAMP8 mice have been used by researchers to connect treatments focused on neuropathological abnormalities with improvements in physiologic functions such as learning and memory acquisition. The most relevant pathological defects found in SAMP8 mice will be discussed in this section.
In the section bellow, we will consider the main features that are known to occur early in the pathogenesis of AD and which the SAMP8 strain exhibits, such as increased oxidative stress, amyloid-β alterations and tau phosphorylation. Although not commented upon here, other features that have been described in AD pathogenesis have been also found in SAMP8 model as: gliosis (Lu et al., 2009), protein alterations in neurons and astrocytes (Diez-Vives et al., 2009), lower hippocampal PKC activity (Hung et al., 2001), and altered protein expression in the olfactory system (Poon et al., 2005c).

## 3. Pathological findings in SAMP8 mice

The unique characteristics of SAMP8 mice are not new, but recently this strain has drawn the attention of researchers [1] in the area of gerontological study of dementias as a result of its exclusive development of learning and memory deficits with age (Flood & Morley, 1998) and as a consequence of the clinical relevance obtained employing other commonly used AD models. In this section, we will summarize recent findings related to pathological data obtained from the SAMP8 strain that are also common in the pathophysiology of AD: damage induced by oxidative stress, Aβ deposition and hyperphosphorylation of tau protein.

### 3.1 Oxidative stress

Free radical-mediated damage to neuronal membrane components has been implicated in the etiology of Alzheimer's disease (AD) and aging; in fact, as has been previously noted, one of the earliest events to occur in AD is oxidative stress (Nunomura et al., 2001). Furthermore, loss of functional enzyme activity during aging had previously been associated with oxidative modification of enzymes (Oliver et al., 1987). Early studies focusing on the oxidative stress level of SAMP8 mice detected elevated levels of lipid peroxide and protein carbonyl in cerebral cortex of SAMP8 mice as young as 4 to 8 weeks old, compared to those of SAMR1 controls (Sato et al., 1996b). After this discovery, this group also confirmed that the oxidative events could be triggered by the loss of activity of anti-oxidative enzymes, such as catalase, and/or by the increase of activity of pro-oxidative enzymes, such as acyl-CoA oxidase (Sato et al., 1996a). These were the first reports that supported the fact that oxidative stress is one of the earliest events related to AD pathology that happen in the SAMP8 strain, and they lead to later papers that studied oxidative stress as a loss of functionality of anti-oxidative enzymes or as an increase in functionality of pro-oxidative enzymes.

[1] Searching for "senescence-accelerated mouse" in PubMed database reported 401 citations on March, 25 2011.

Other authors have complemented these studies using more powerful approaches, such as proteomics, to study the level of carboxylation of proteins in the SAMP8 strain. Therefore, higher protein oxidation and lipid peroxidation reported by Sato et al. were confirmed in 12-month-old SAMP8 mice (Poon et al., 2004a). Those oxidative stress markers were reduced not only when SAMP8 mice were treated with antioxidants, such as α-lipoic acid (Poon et al., 2005b), as would be expected, but also when antisense therapy directed to decrease APP expression was carried out (Poon et al., 2004b, 2005a). Both approaches are capable of reversing cognitive deficits in 12-month-old SAMP8 mice. Furthermore, a complete proteomics study including about 1700 proteins was carried out in SAMP8 and SAMR1 mice (Zhu et al., 2011). In this report there was found to be a group of proteins which were expressed in an age-dependent way and, interestingly, some of them had previously been found to be expressed in AD patients. One of these, Cu/Zn superoxide dismutase (De Leo et al., 1998), will be commented upon later in this chapter. Taken together, these reports related to oxidative stress have not only provided understanding of the mechanisms underlying memory and learning deficits but have also suggested potential therapies to counteract age-related dementia. Therefore, it has been demonstrated for the SAMP8 strain that age-related dementia observed in these animals is ROS-dependent, as the treatment with antioxidants improved learning and memory ability and reduced Aβ deposition (Shih et al., 2010).

Several studies have been carried out in the SAMP8 strain focused on physiologic enzymes with antioxidant properties that counteract ROS produced by oxidative metabolism, or on enzymes with pro-oxidant properties that support ROS production. The first of these was carried out a long time ago when SAM nomenclature had not yet been established. In this study, there was reported to be elevated activity of monoamine oxidase B (MAO-B) and decreased activity of superoxide dismutase (SOD) in SAM-P mice versus SAM-R mice (Nomura et al., 1989). After that, more detailed studies were performed specifically in the SAMP8 strain where there was reported to be a decrease in manganese superoxide dismutase (Mn-SOD) activity in 10-month-old SAMP8 mice (Kurokawa et al., 2001), and an age-related decrease in Cu/Zn superoxide dismutase (Cu/Zn-SOD) expression in 5 to 15-month-old SAMP8 mice (Zhu et al., 2011), as well as in activity of glutathione peroxidase in 12-month-old SAMP8 mice (Okatani et al., 2002). Furthermore, glutathione reductase, glutathione peroxidase and catalase activities were lower in 5-month-old SAMP8 mice than in aged-matched SAMR1 mice (Sureda et al., 2006).

Another family of enzymes involved in ROS damage is nitric oxide synthases (NOS) composed of three isozymes. Nitric oxide (NO) is a signaling molecule widely distributed in the nervous system whose increase has been related to a variety of neurodegenerative pathologies such as AD (Steinert et al., 2010). In SAMP8 mice an age-related increase of NOS activity has been reported which can be reduced by natural antioxidants (Inada et al., 1996). This age-related increase in NOS activity was not due to an increase in mRNA or protein levels (Ali et al., 2009). Although it has been previously reported that Aβ antisense treatment reduced oxidative stress markers (Poon et al., 2004b), recent studies suggest that regulation of the NOS family is complex, as Aβ antisense or antibody treatment further increased NOS activity, with an increase in inducible NOS (iNOS), and reduced neuronal NOS (nNOS). No changes were reported in endothelial NOS (eNOS) (Ali et al., 2009).

A summary of the reported variations in enzymatic activity of anti and pro-oxidant enzymes in the SAMP8 strain is presented in Table 2.

| **Anti-oxidative enzymes (decreased oxidative stress)** | **Pro-oxidative enzymes (increased oxidative stress)** |
|---|---|
| Catalase ↓ | Acyl-CoA oxidase ↑ |
| Manganese superoxide dismutase ↓ | Monoamine oxidase B ↑ |
| Cu/Zn superoxide dismutase ↓ | NOS ↑ |
| Glutathione peroxidase ↓ | |
| Glutathione reductase ↓ | |

Table 2. Summary of the variations reported in enzymes related to oxidative stress in the SAMP8 strain.

Some antioxidants, such as melatonin, have been used in SAMP8 mice to counteract oxidative damage. The effect of melatonin treatment on the activity of AD-related kinases has been described (Gutierrez-Cuesta et al., 2007). In addition, melatonin-treated SAMP8 mice reported elevated levels of activity of glutathione peroxidase, an anti-oxidant enzyme, improving indexes of lipid peroxidation and oxidative stress by a combination of melatonin scavenger activity and its ability to stimulate anti-oxidant enzymes (Okatani et al., 2002). Furthermore, as there is clear evidence of the relationship between oxidative stress and age-related dementias in SAMP8 strain, these mice have also been used to test the effects of some natural antioxidants in the diet. Natural antioxidants derived from plants, such as rose-flower extract, have been tested as antioxidant compounds in the SAMP8 strain. In this case, it was demonstrated that antioxidant treatment increased activity and expression of the anti-oxidant enzymes catalase and glutathione peroxidase, decreasing lipid peroxidation and therefore extending the lifespan of SAMP8 (Ng et al., 2005). The antioxidant effects of other plant extracts, such as Toona sinensis Roemor, have been also tested in the SAMP8 strain. The results obtained demonstrated that dietary supplemented animals presented decreased Aβ deposition and lower levels of antioxidant markers as well as higher levels of anti-oxidant enzymes, such as SOD, catalase and glutathione peroxidase (Liao et al., 2006). Recently, icariin, a flavonoid extracted from several plant species, was shown to improve age-related deficits in SAMP8 mice by decreasing NO levels and NOS activity, and by increasing glutathione peroxidase and SOD activities (He et al., 2010). Finally, the administration of lotus seedpod proanthocyanidins to SAMP8 mice decreased NO levels and lowered NOS activity while increasing glutathione peroxidase and SOD activity, which correlates whit an improvement in SAMP8 cognitive deficits (Gong et al., 2008)

Nevertheless, the literature related to oxidative stress in SAMP8 strain is not always conclusive. For example while Okatani et al. did not find age-related changes in SOD, either in SAMP8 or in SAMR1 strain (Okatani et al., 2002), Alvarez-Garcia et al. did not observe any age-related differences in activity of catalase and glutathione reductase, while they did in SOD (Alvarez-Garcia et al., 2006). Finally, Sureda et al. found decreased activity of glutathione reductase, glutathione peroxidase and catalase in SAMP8 mice (Sureda et al., 2006). In addition, other systems might also participate in brain oxidative stress processes. Recent studies have demonstrated that, as in AD brain, peroxide detoxification by astrocytes could play a role in age-related cognitive deficits. Astrocytes derived from SAMP8 mice were more susceptible to $H_2O_2$ induced damage than those derived from SAMR1 mice. Decreased peroxide detoxification was seen in SAMP8 mice (Lu et al., 2008). Taken together, these results remind us that there is still a long way to go before we completely understand

the role of oxidative stress in SAMP8 age-related cognitive deficits and, by extension, in AD pathology.

### 3.2 Amyloid-β deposition

Senile plaques are one of the hallmarks of AD and have become one of the main hypotheses for the development of this disease (Selkoe, 2000). This hypothesis is based on the fact that familial AD (≈5% of AD cases) may be caused by mutations in the amyloid-β precursor protein (APP) or by mutations in genes that participate in amyloid-β cleavage (presenilins-1/2). According to this hypothesis, the increase in Aβ peptide, which would result in an increase in the number of senile plaques, is the main factor that triggers the rest of the clinical and histopathological features reported in AD. Although this hypothesis has not been refuted, it has not been demonstrated, despite the vast knowledge acquired from models and clinical studies, and even most current reviews on this topic continue to include the words "hypothesis" or "speculation" (Marchesi, 2011).

Although the SAMP8 strain has been proposed as a model of AD, the chronology of appearance of Aβ deposits has been controversial for a long time. Furthermore, there was one main difference between these deposits and the ones found in humans: SAMP8 Aβ deposits were not stained using Congo red and Thioflavin S (Takemura et al., 1993), a classic method used to stain amyloid plaques (Kelenyi, 1967). This main difference has led some researchers to incorrectly consider the age-related dementia described in SAMP8 mice as independent of amyloid plaque deposition (Ashe & Zahs, 2010). In one of the first studies of Aβ deposition in SAMP8 strain, the authors Takemura et al. proposed that little differences in the primary structure of APP or in the processing enzymes could lead to different processed proteins that might be responsible for their different polymerization into the β-pleated sheet. Although possible, this explanation seems improbable since it has been demonstrated that SAMP8 APP protein shows high similarity to that of other species (99.2% homologous with that of mouse and rat (Kumar et al., 2001)). In addition, since its discovery in 1967, the staining method based on fluorophores, such as Congo red and Thioflavin S, has been modified in order to increase the resulting resolution (Sun et al., 2002).

Nevertheless, Aβ deposits in SAMP8 strain have been detected in AD-affected regions (including the hippocampus, medial septum, cerebral cortex and cerebellum) using a wide variety of antiserum, although until a few years ago the date of appearance of Aβ deposits was open to question. One of the first studies to demonstrate the appearance of amyloid plaques in young SAMP8 mice was published in 2006 (Liao et al., 2006), but it was not until 2010 that a complete study was carried out. In this research, Del Valle et al. performed a time course of Aβ deposition in SAMP8 strain, demonstrating that amyloid plaques could be observed in the hippocampus of 6-month-old SAMP8 mice and that they increased with age, while 15 months was needed to observe them in control SAMR1 mice (Del Valle et al., 2010).

As well as immunodetection, quantitative expression studies of AD-related genes were carried out. All this research points to an increase in Aβ levels in the SAMP8 strain. In this sense, an age-related increase of expression of APP gene in SAMP8 hippocampus has been detected which correlated with an age-related increase in APP protein levels (Morley et al., 2000; Poon et al., 2004a). In agreement with these findings, variations in the expression of other genes related to AD pathology have also been reported. In this sense, there have been studies of the expression of presenilin-1 (PS1) and presenilin-2 (PS2), two proteins that are included in the γ-secretase complex whose mutations give rise to an increase in

amyloidogenic cuts of APP observed in familial AD. In the SAMP8 strain an increase in the gene expression of both PS1 and PS2 has been reported (Wei et al., 1999; Kumar et al., 2009) which may be one of the causes of increased processing of APP that leads to Aβ accumulation. The expression of another familial AD marker, apolipoprotein E (ApoE), was also studied in the hippocampus of the SAMP8 strain (Wei et al., 1999). This biomarker was less expressed in SAMP8 mice compared to the SAMR1 strain. In contrast, ApoE allele ε4 expression is associated with increased susceptibility to AD. Although, the connection between AD and ApoE is not clear, some researchers postulate that ApoE could be the link between Aβ deposition and pathological alterations of the cerebral microvasculature in AD (Marchesi, 2011). Apolipoprotein A-II (ApoAII) is the precursor protein that induces mouse senile amyloidosis, a form of amyloidosis in which the severity of Aβ deposition increases with age. This has also been the focus of research in SAMP8 strain which expresses type A ApoAII, considered as moderately amyloidogenic (Higuchi et al., 1991). This finding made the SAMP8 strain an appropriate model system for the study of the mechanism(s) of age-related amyloid fibril formation. In conclusion, all the foregoing research suggests that every one of these findings could contribute to the age-related Aβ accumulation described in the SAMP8 strain.

In addition to all the data already considered, there are other factors that contribute to Aβ accumulation. Among these is the Aβ peptide rate of efflux from the brain to blood. There are some hypotheses concerning how this peptide could be transported across the blood brain barrier which will not be discussed here. What is clear, however, is that the higher the efflux rate of Aβ is, the less Aβ remains inside the central nervous system where it may accumulate. This phenomenon was studied by Banks et al., using radioactive labeled Aβ which was centrally administered to SAMP8 mice. This group demonstrated that Aβ efflux was impaired in SAMP8 mice, suggesting that Aβ-impaired efflux could be an early contributor to Aβ deposition in the SAMP8 strain (Banks et al., 2003).

As noted above, if the hypothesis that Aβ deposition is responsible for the cognitive/behavioral deficits observed in SAMP8 strain holds, then when Aβ accumulation is inhibited, independently of the technique employed, the reported cognitive and behavioral deficits should improve. This hypothesis is correct for APP inhibition in SAMP8 strain and has been validated by several reports. Some of these have been commented upon earlier in this chapter but the original work in which downregulation of APP was achieved using a specific oligonucleotide was performed by Kumar et al. (Kumar et al., 2000). After this report, other authors demonstrated that APP expression could be decreased using antisense APP oligonucleotides (Poon et al., 2004b), improving learning and reversing memory deficits. Recently, a new strategy, employing micro ribonucleic acids (miRNAs), which are post-transcriptional regulators of APP in SAMP8 mice and show different expression patterns in SAMP8 and SAMR1 mice, has been proposed (Liu et al., 2010b). These authors hold that as miRNAs (miR-16, miR-144, miR-195, and miR-383) negatively regulate APP expression in SAMP8 strain, the use of these miRNAs could help to improve cognitive deficits in the SAMP8 strain, although this hypothesis has not been tested yet. Furthermore, Aβ immunotherapy strategies were employed as well using Aβ-directed antibodies, injected intracerebroventricularly (Morley et al., 2002) or intraperitoneally (Zhang et al., 2011), with recovery in cognitive deficits in reported in the SAMP8 strain.

Although Aβ accumulation is associated with cognitive impairment, there are some animal models of AD whose age-related brain disorders are not related to amyloid plaques but rather to abnormal APP metabolism that could accelerate age-related brain disorders

independently of Aβ deposition (Ashe & Zahs, 2010). As can be deduced from all the evidence described here, this is not the case with the SAMP8 strain. In addition, there was a study recently carried out using a monoclonal antibody against Aβ oligomers on SAMP8 mice which makes it increasingly evident that the age-related deficits observed in SAMP8 strain are Aβ deposition-dependent. In this study, the administration of this specific antibody to SAMP8 mice improved learning and memory tasks and decreased Aβ oligomers and phospho-tau levels (Zhang et al., 2011).

### 3.3 Hyperphosphorylation of tau

The presence of neurofibrillary tangles, consisting of hyperphosphorylated tau protein, is another hallmark of AD, although formation of neurofibrillary tangles is considered a consequence of Aβ accumulation (Hardy & Selkoe, 2002). Tau is a highly soluble protein which is implicated in microtubule assembly and stabilization whose 6 known cerebral isoforms are translated from a single gene by alternative splicing. Although tau mutations have been involved in a variety of neurodegenerative disorders named familial tauopathies (Goedert et al., 1998), tau is not mutated in AD. Different tau functions are controlled by site-specific phosphorylation which affects microtubule binding. While kinase-mediated phosphorylation inhibits microtubule binding, phosphatase-mediated dephosphorylation restores microtubule binding. In AD pathology, aberrant tau hyperphosphorylation induces microtubule instability and promotes a toxic effect on neurofibrillary tangles surrounding neurons (Johnson & Stoothoff, 2004). Therefore, if the SAMP8 strain is a valid animal model for the study of AD, the pathology induced by tau phosphorylation should be similar in AD and in SAMP8 mice.

In contrast to Aβ accumulation, total tau quantity is not a critical factor in toxicity, as is kinase-mediated phosphorylation. Consequently, an increase in phosphorylated tau in SAMP8 (cortex, striatum and hippocampus, and to a lesser extent in cerebellum) has been detected compared with SAMR1, whilst levels of tau protein were maintained overall in the different areas (Canudas et al., 2005). Furthermore, elevated levels of phosphorylated tau protein were found in SAMP8 strain at an early age, in 5-month-old mice (Alvarez-Garcia et al., 2006), indicating that tau phosphorylation is an early event in the SAMP8 strain. Thus, the kinase(s) responsible(s) for the phosphorylation of tau protein in the SAMP8 strain needed to be studied in detail. Two of the serine/threonine kinases which phosphorylate human tau protein are cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 (GSK3). When they were studied in detail, an elevated activity of CDK5 accompanied by an increase in expression was detected in cortex, striatum, hippocampus and, a lesser extent, in cerebellum (Canudas et al., 2005). However, GSK3β expression and activity were not altered in SAMP8 mice (Canudas et al., 2005). Yet chronic melatonin treatment in SAMP8 mice diminished tau hyperphosphorylation, reducing CDK5 and GSK3β activation and suggesting that these kinases and their downstream pathways participate in tau hyper-phosphorylation in SAMP8 mice (Gutierrez-Cuesta et al., 2007).

Besides the relationship between tau protein and AD-related kinases, a relationship that links oxidative stress and tau phosphorylation has also been established. We noted earlier the work of Gutierrez-Cuesta et al., which links chronic antioxidant treatment with reduced tau phosphorylation, probably due to a reduction in CDK5/GSK3β activation, in SAMP8 mice. This relationship is attested by the abundant bibliography that supports the antioxidant properties of lithium. Lithium treatment in SAMP8 mice decreased CDK5/GSK3β activation, reducing tau phosphorylation and providing neuroprotection to

SAMP8 mice (Tajes et al., 2008). However, to date GSK3 has not been seen to be involved in the antioxidant properties of lithium (Camins et al., 2009). All the data corroborates a chronology of appearance of AD-like pathology in the SAMP8 strain: as oxidative stress is considered an early event in AD, the use of antioxidants should improve subsequent events in this pathology.

Recently, it has been postulated that elevation of endogenous formaldehyde levels may be related to the pathogenic processes of neurodegenerative diseases. From this, it has been demonstrated that some aldehydes, directly related to lipid peroxidation, are not only able to enhance amyloid plaque formation (Chen et al., 2006), but also to induce misfolding of tau protein and formation of globular amyloid-like aggregates (Nie et al., 2007). Thus, in addition to all the available research pointing to SAMP8 as an appropriate model for the study of age-related disorders, it has been recently described that formaldehyde levels are significantly increased in the SAMP8 strain (Tong et al., 2011), which suggests that endogenous formaldehyde is related to aging and that SAMP8 strain could be a suitable system for the study of these neurotoxic aggregates that could play a role in the induction of tauopathies.

## 4. Neurochemical changes in SAMP8 mice: GPCR mediated pathways

G protein-coupled receptors (GPCRs) are a large superfamily of membrane-bound signaling proteins that are involved in the regulation of a wide range of physiological functions and which constitute the most common target for therapeutic intervention (Ma & Zemmel, 2002). Visual, olfactory and taste sensation, intermediary metabolism, cell growth, differentiation and many other phenomena are all influenced by GPCR signals (Luttrell et al., 2008). GPCR had been viewed as a simple on-off switch that used a single class of effector molecule: the heterotrimeric G proteins. However, converging lines of evidence demonstrate the existence of G protein-independent signal transduction and its unique biochemical and physiological effects (Rajagopal et al., 2010). This increasing complexity of molecular mechanisms responsible for GPCR signaling and its regulation, through interplay of positive and negative regulatory events that amplify the effect of a hormone binding the receptor or that dampen cellular responsiveness, should be tackled as a very promising field to develop new therapeutic approaches. Many G protein-coupled receptors and/or their corresponding signaling pathways, such as cholinergic, glutamatergic, serotonergic, adrenergic and peptidergic neurotransmitter systems, are deregulated in brain from AD cases. This deregulation could be responsible for an altered sequential cleavage of APP by the α-, β-and γ-secretases, which are regulated by GPCRs, and the determination of the extent of amyloid-β peptide generation. In turn, amyloid-β can directly or indirectly affect GPCR function (for a review see Thathiah & De Strooper, 2011). Different neurochemical changes have also been found in the SAMP8 brain, including modifications in signal transduction pathways mediated by G protein-coupled receptors, ion channel receptors and nuclear hormone receptors. These changes have been reported at different levels (e.g. neurotransmitter concentration, receptor number and/or affinity, second messenger generation). Several GPCRs have been analyzed in the SAM model. However, many available data deal with the effects on learning and memory deficits in SAMP8 mice due to the administration of different ligands with agonist activity on these receptors. In contrast, very few reports have tried to analyze the possible modulation of the receptor itself (i.e., quantity, affinity,

regulation, etc.). Available data on different GPCR pathways analyzed in AD and the SAMP8 model are summarized in Table 3.

### 4.1 Serotonin receptors

The 5-hydroxytryptamine (5-HT; serotonin) receptors belong to both the G protein-coupled and ligand-gated ion channel superfamilies. These have been divided into seven distinct families, or classes, according to structural diversity and the preferred effector mechanism. Some of these classes comprise multiple receptors, which share similar structural and effector properties while displaying very different operational profiles (Barnes & Sharp, 1999). The serotonergic system has been implicatred in learning and memory (Reis et al., 2009). A possible age-related change in serotonin (5-HT) receptor antagonist dose–response curves was analyzed in SAMP8 mice using a retention test on a T-maze footshock avoidance apparatus (Flood et al., 1996, 1998). 5-$HT_1$ and 5-$HT_2$ receptor antagonists, methiothepin and ketanserin, improved retention, but the dose needed to improve retention was at least 10 times greater in 12-month-old P8 mice than in 4-month-old mice, indicating that there was a reduction in serotonin receptor activity in the 12-month-old mice. Similar drug studies done in the hippocampus of 4- and 12-month-old mice found no age-related change in the dose–response curves of serotonin antagonists for improvement of retention (Flood et al., 1996). Therefore, these results suggest that with increasing age SAMP8 develops a dysfunction in septohippocampal functioning which may initially begin with a decrease in serotonergic activity that appears to be specific to the septum. In agreement with this, the serotonin uptake inhibition by indeloxazine hydrochloride [(±)-2[(inden-7-yloxy)methyl]morpholine hydrochloride], a cerebral metabolic enhancer with cerebral activating properties (Yamamoto, 1990), potentiated the serotonergic system in SAMP8 mice (Yamaguchi et al., 1998). In fact, 5-hydroxytryptamine (5-HT) levels are significantly lower in SAMP8 as compared to SAMR1 mice (Qiu et al., 2010). Similar serotonergic deficits have been reported in patients with AD, including loss of 5-$HT_2$ receptors in cerebral cortex (Blin et al., 1993), 5-$HT_4$ in hippocampus and frontal cortex (Reynolds et al., 1995) and 5-$HT_6$ in pyramidal cells (Lorke et al., 2006).

### 4.2 Acetylcholine receptors (muscarinic)

Muscarinic receptors mediate cellular response to the natural ligand acetylcholine (ACh). They enjoy widespread tissue distribution and are involved in the control of numerous central and peripheral physiological responses, as well as being a major drug target in human disease. This family of G protein-coupled receptors consists of five members designated $M_1$-$M_5$. Hence, $M_2$ and $M_4$-muscarinic receptors are able to couple to the pertusiss-toxin sensitive $G_{i/o}$ proteins, and $M_1$, $M_3$ and $M_5$-muscarinic receptors couple to $G_{q/11}$ proteins. However, the muscarinic receptor family can couple to a wide range of diverse signaling pathways, mediated or not by G proteins (Caulfield & Birsall, 1998; van Koppen & Kaiser, 2003). In the hippocampus of SAMP8 at 12 months, binding of [$^3$H]pirenzepine, a $M_1$ muscarinic receptor antagonist, was significantly lower than that in SAMR1. However, in the cerebral cortex, binding was higher in SAMP8 than in SAMR1 at 12 months (Nomura et al., 1997). In addition, SAMP8 mice show decreased release of acetylcholine (Qiu et al., 2010). Thus, there is a decreased cortical acetylcholine content in SAMP8 (Kitaoka et al., 2010). Moreover, there is a reported effect of dysfunctional teeth on age-related changes in the septohippocampal cholinergic system by assessing acetylcholine

(ACh) release and choline acetyltransferase (ChAT) activity in the hippocampus of young-adult and aged SAMP8 mice after removal of their upper molar teeth (molarless condition) (Onozuka et al., 2002). In this experimental model, significantly less KCl-evoked ACh release was seen in aged molarless SAMP8 mice compared with aged controls, whereas the molarless condition had no effect on young mice. In the control groups, the KCl-evoked ACh release decreased with aging, but the difference was not statistically significant. Moreover, in control mice, ChAT activity was higher in the young-adult group than in the aged mice, indicating an age-dependent decrease in hippocampal ChAT activity in SAMP8 mice. In addition, aged molarless mice showed a greater reduction in ChAT activity than age-matched control mice, whereas the molarless condition had little effect on young-adult mice (Onozuka et al., 2002). This decreased cholinergic activity results in a decreased ability of the SAMP8 mouse to learn and retain new information (Morley et al., 2002). In fact, $M_1$ mAChR is the most abundant subtype in the cortex and hippocampus, two major brain regions that develop amyloid plaques and neurofibrillary tangles (nFTs) in AD, and postsynaptic $M_1$ mAChRs play a major role in hippocampus-dependent short-term memory and memory consolidation (Anagnostaras et al., 2003), which is impaired in AD (Levey, 1996). Therefore, considerable efforts have been directed towards developing $M_1$ mAChR-selective agonists that are capable of restoring the cognitive deficits in patients with AD, in whom hippocampus $M_1$ level are reduced (Pakrasi et al., 2007) while they are increased in frontal cortex (Svensson et al., 1992).

### 4.3 Adenosine receptors

Adenosine is a nucleoside widely distributed in central and peripheral nervous system that exerts its actions through four types of receptors named $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$, all of them being GPCRs. $A_1$ and $A_3$ receptors are coupled, through $G_{i/o}$ proteins, to adenylyl cyclase activity inhibition, while $A_{2A}$ and $A_{2B}$ receptors are coupled to stimulation of the enzymatic activity, through $G_s$ protein (Ralevic & Burnstock, 1998; Fredholm et al., 2001, 2005). Out of the four adenosine receptors, the $A_1$ 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 & Masino, 2001). $A_{2A}$ receptors are concentrated in the basal ganglia but they are also present throughout the brain, albeit in a considerably lower density. $A_{2B}$ and $A_3$ receptors are the least abundant in the brain (Cunha, 2005). Adenosine receptor gene expression and the quantification of $A_1$ and $A_{2A}$ proteins in plasma membranes were analyzed recently in SAMP8 and SAMR1 strains using 21- and 180-day-old animals (Castillo et al., 2009). Results show that mRNA coding adenosine $A_1$ and $A_{2B}$ receptors is significantly increased in middle-aged versus young SAMR1 animals, suggesting an age-associated up-regulation in resistant control mice. However, these increases were not detected in SAMP8 animals. Concerning $A_{2A}$ receptors, no significant differences were found between young and middle-aged animals, either in SAMP8 or in SAMR1. However, the level of $A_{2A}$ mRNA expression was significantly lower in SAMP8 versus SAMR1. Finally, an increase in mRNA coding $A_3$ receptor was observed in both middle-aged SAMR1 and SAMP8 animals without significant differences between strains. Adenosine $A_1$ receptors were measured in plasma membranes from SAMR1 and SAMP8 mice by binding assay using [$^3$H]DPCPX, a selective $A_1$ receptor antagonist, as radioligand. Total adenosine $A_1$ receptor numbers in middle-aged SAMR1 decreased by 38%, suggesting a loss of receptors associated with aging. This decrease was not observed in

the SAMP8 strain. However, and of interest, total $A_1$ receptor level in SAMP8 was significantly lower than in SAMR1, with even fewer receptors detected than in middle-aged SAMR1 mice, suggesting that $A_1$ 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. This age-related loss of $A_1$ receptors in SAMR1 was then 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 $A_{2A}$ receptors was observed in SAMR1 with no variation in SAMP8, by Western-blotting. The most important finding in the paper of Castillo et al. is that adenosine $A_1$ 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. Higher $A_1$ and $A_{2A}$ receptor expression levels have been found in the frontal cortex of post-mortem brains of patients with AD (Albasanz et al., 2008). Interestingly, $A_{2A}$-deficient mice display improved spatial recognition memory (Wang et al., 2006), whereas in vivo $A_{2A}$ overexpression leads to memory deficits (Gimenez-Lort et al., 2007). In agreement, pharmacological blockade of $A_{2A}$ receptors by caffeine seems to reduce Aβ formation and the risk of developing dementia (Chen & Chern, 2011; Gelber et al., 2011).

### 4.4 Adrenergic receptors

Adrenoceptors are 7-transmembrane receptors which mediate the central and peripheral actions of the neurotransmitter noradrenaline (norepinephrine), and the hormone and neurotransmitter adrenaline (epinephrine). Adrenoceptors are found in nearly all peripheral tissues and on many neuronal populations within the central nervous system. Both noradrenaline and adrenaline play important roles in the control of blood pressure, myocardial contractile rate and force, airway reactivity, and a variety of metabolic and central nervous system functions. Based on both pharmacological and molecular evidence they are classified into three major types -$\alpha_1$, $\alpha_2$ and β- each of which is further divided into at least three subtypes (Bylund et al., 1994). Agonists for norepinephrine receptors required little or no change in the dose needed to improve retention in older SAMP8 (12- versus 4-month-old) mice on a T-maze footshock avoidance test (Flood et al., 1998), suggesting that this signaling pathway is not modified with aging in SAMP8 mice. In contrast, the level of total β-adrenergic receptors and, more interestingly, the relative ratio of $\beta_1$-/$\beta_2$-receptors, have been reported as altered in different brain regions from AD patients (Kalaria et al., 1989). Thus, there is an increase in $\beta_2$-receptors in prefrontal cortex and hippocampus which could be responsible for the amelioration in AD patients detected by blocking β-adrenergic receptors with selective antagonists (Yu et al., 2011). Thus, a similar modulation in SAMP8 mice must not be ruled out as no detailed studies have been performed to date.

### 4.5 Calcitonin receptors

The calcitonin peptide family comprises calcitonin, amylin, calcitonin gene-related peptide (CGRP), adrenomedullin (AM) and AM2, also known as intermedin. All of these peptides, ranging from 32 to 52 amino acids in length in humans, share structural similarities, and their truncation beyond the second cysteine residue generates antagonists. With the

exception of CGRP receptors, such modified forms of the native peptides are the only pharmacological tools currently available for characterizing these receptors. The receptor family for these peptides consists of two class B GPCRs, the calcitonin receptor (CR) and calcitonin receptor-like receptor (sometimes abbreviated as CRLR) for which pharmacological specificity is dictated by additional proteins, known as receptor activity-modifying proteins (RAMPs). These are integral parts of the receptor complex (Hoare et al., 2005). In male SAMP8 and SAMR1 mice, blood samples were collected monthly from 3 to 12 months of age. With advancing age, the plasma calcitonin (CT) levels decreased progressively in both SAMR1 and SAMP8. However, the curve of age-related changes in the plasma CT levels was lower in SAMP8 than in SAMR1 (Chen et al., 2004). Measurement of CT level in female SAMP8 mice at the age of 3, 6, 9, 12, and 15 months revealed that plasma CT level decreased with aging and/or ovariectomy. Plasma CT levels in the ovariectomized (Ovx) mice were significantly lower than those in the intact mice. In addition, the decrease of plasma CT level was moderated by long-term dietary antler supplementation in both Ovx and intact mice (Chen at al., 2007). Although no data were found concerning CT receptors in SAMP8 mice, a reduced level of the endogenous agonist (CT) could suggest reduced signaling through these receptors. In fact, a possible receptor upregulation could be expected in response to low CT levels as a compensatory mechanism. Interestingly, the amylin receptor is a putative target for the actions of Aβ in the brain. Thus, in primary cultures of human fetal neurons (HFNs), AC253, an amylin receptor antagonist, blocks the electrophysiological effects of Aβ. Moreover, in transgenic mice (TgCRND8) that overexpress amyloid precursor protein, amylin receptor is up-regulated in specific brain regions that also demonstrate an elevated amyloid burden (Jhamandas et al., 2011).

### 4.6 Chemokine receptors

Chemokines (chemotactic cytokines) comprise a family of related proteins according to structural criteria including overall amino acid sequence homology, length, conserved cysteine motifs and a common fold. They are involved in leukocyte trafficking, antimicrobial activity, HIV inhibitory activity, angiogenic or angiostatic activity, tumour-promoting or tumour-inhibiting activity, apoptosis or mitogenic activity, and the ability to modulate gene expression, T cell differentiation and phagocyte activation. Chemokines act by binding to 7-transmembrane domain, G protein-coupled receptors. There are 18 human chemokine receptors and over fifty distinct chemokines (Melik-Parsadaniantz & Rostene, 2008). An interesting region has been found on chromosome 4 of SAMP8 mice harboring multiple genes that were more highly expressed in SAMP8 than in SAMR1; some of these genes are chemokine (C-C motif) ligand 19 (Ccl19) and Ccl27. The RNA levels for these two genes in retina or hippocampus were higher in SAMP8 than SAMR1 (Carter et al., 2005). In addition, CCL2, a small cytokine belonging to the CC chemokine family that is also known as monocyte chemotactic protein-1 (MCP-1), has a significantly increased expression in old as compared with young SAMP8 mice, and this expression was significantly reduced after melatonin treatment. In SAMR1 mice no statistically significant differences between young and old animals were found. CCL2 expression was also elevated in old SAMP8 mice when compared with old SAMR1 mice (Cuesta et al., 2010). CCL2 is the main ligand for chemokine receptor CCR2, which is required for macrophage infiltration at sites of axonal injury in the hippocampus. CCL2 has been localized to mature amyloid plaques in the AD brain (Ishizuka et al., 1997).

### 4.7 Dopamine receptors

Dopamine receptor subtypes are named $D_1$-$D_5$ and belong to two subfamilies, $D_1$-like and $D_2$-like, based upon similarities in sequence, pharmacology and ability to stimulate or inhibit adenylyl cyclase activity mediated via coupling to $G_{\alpha s}$ or $G_{\alpha i/o}$ proteins (Beaulieu & Gainetdinov, 2011). Retention improvement by agonists for dopamine receptors was similar in older SAMP8 (12- versus 4-month-old) mice on a T-maze footshock avoidance test (Flood et al., 1998), suggesting that this signaling pathway is not modified with aging in SAMP8 mice. However, there is a decrease in the number of dopamine (DA) neurons and the associated ultrastructural changes in the neurons of the nigrostriatal system in SAMP8 as compared with SAMR1. This reduction is even greater in aged SAMP8 mice (8–10 months old) (Karasawa et al., 1997), suggesting a reduced DA level in SAMP8 worsened by age. The administration of 2,4-diamino-6-hydroxypyrimidine (DAHP), an inhibitor of GTP cyclohydrolase I, to inhibit DA and serotonin syntheses in young mice (2 months old) and aged mice (10 months old), revealed that DA turnover is lower in aged mice than in young mice (Karasawa et al., 1999). This DA level could be modulated by diet or MPTP administration. DA level was similarly and significantly increased in cerebellum at 8 and 12 months of age after diet restriction (Kim & Choi, 2000). In contrast, administration of MPTP can induce a marked decrease in striatal DA levels and a loss of dopaminergic neurons in the substantia nigra of SAMP8 mice (Liu et al., 2008). Moreover, old MPTP-SAMP8 mice show an earlier and more severe loss of dopaminergic neurons than young mice (Liu et al., 2010a). No data concerning modulation of dopamine receptors was found for SAMP8 mice. However, in frontal cortex from AD brain, expression of $D_1$, $D_3$ and $D_4$ receptors was severely reduced, $D_2$ was moderately reduced and $D_5$ was the only receptor subtype whose expression was increased in AD (Kumar & Patel, 2007). Also, in temporal lobe of AD brain these dopamine receptors have a reduced expression (Gahete et al., 2010). In addition, DA plasma level is significantly lower in AD patients (Umegaki et al., 2000).

### 4.8 $GABA_B$ receptors

The $GABA_B$ receptor is a $G_i/G_o$ protein-coupled receptor heterodimer with two subunits, designated 1 and 2. Activation at this site produces neuronal hyperpolarization by increasing membrane $K^+$ conductance. The antispastic agent (-)-baclofen is a highly selective agonist for $GABA_B$ receptors (Martin et al., 2001). Saclofen, a $GABA_B$ receptor antagonist, had to be injected into the septum at a higher dose in 12- than in 4-month-old mice to improve retention in SAMP8 mice after training on footshock avoidance (Flood et al., 1998), suggesting a possible upregulation of $GABA_B$ receptor. However, a quantitative autoradiography study of $GABA_B$ and $GABA_A$ receptors in AD revealed fewer hippocampal $GABA_B$ receptors in stratum moleculare of the dentate gyrus, stratum lacunosum-moleculare and stratum pyramidale of CA1 (Chu et al., 1987a), and a significantly lower $GABA_B$ level in frontal cortex (Chu et al., 1987b), with no significant differences on affinity (Kd). Also, the GABA level in cerebrospinal fluid was significantly reduced in AD patients (Jimenez-Jimenez et al., 1998).

### 4.9 Melatonin receptors

There are three major plasma membrane receptors for melatonin within the brain. Two of these, $MT_1$ and $MT_2$, are GPCRs whose activation leads to decreased levels of cyclic AMP. The third, $MT_3$ or NQO2, is a quinone reductase with poorly understood in vivo function

(Reppert et al., 1996; Rios et al., 2010; Tan et al., 2007). However, other mechanisms of action could be possible as there are additional melatonin binding sites in the nucleus of many cell types (Filadelfi & Castrucci, 1996). Melatonin acts as a neurohormone affecting transcriptional events in the CNS (Kotler et al., 1998). Several studies have shown that melatonin levels are diminished in AD patients compared to age-matched control subjects. CSF melatonin levels decrease even in preclinical stages when the patients do not manifest any cognitive impairment (at Braak stages I-II), suggesting that the reduction in melatonin is an early marker for the first stages of AD (for a review see Cardinalli et al., 2010). $MT_1$ activation depresses CREB and stimulates ERK (Chan et al., 2002). $MT_2$ levels are depressed in AD (Savaskan et al., 2005). Changes in these signaling pathways may form the basis of the alteration in gene expression effected by melatonin. The linkage between reduced melatonin levels and accelerated aging has been reviewed elsewhere (Bondy & Sharman, 2007) and may be more than merely correlative. Levels of both $MT_1$ and $MT_2$ receptors are very high within the suprachiasmatic nucleus (SCN), the site of circadian rhythm regulation. However, although administration of melatonin in drinking water promotes the phase advance of light–dark cycle in senescence-accelerated SAMR1 but not SAMP8 mice, levels of expression of both $MT_1$ and $MT_2$ mRNAs in the SCN are identical in the two SAM strains (Asai et al., 2000).

### 4.10 Opioid receptors

The opioid peptide receptors are heterogeneous. Measures of antagonist affinities against various opioid agonists in different systems resulted in unambiguous evidence for heterogeneity of receptor types, and the eventual definition of the μ, δ and κ receptor types (Dawan et al., 1996). In vivo (+)-[$^3$H]SKF-10,047 binding to σ sites was examined in the hippocampal formation and cerebral cortex of SAMR1 and SAMP8, at 12 months of age. Binding levels expressed as fmol/mg of proteins indicated decreases in the levels of total (+)-[$^3$H]SKF-10,047 bound in the cortex of SAMP8 as compared with SAMR1, but not in the hippocampus, and in the levels of (+)-[$^3$H]SKF-10,047 non-specifically bound in both structures. However, bound-to-free ratios never significantly differed between the two substrains. The apparent reductions in the in vivo binding levels of SKF-10,047 tracer may thus not reflect decreased binding capacities at the σ sites. However, the learning impairment observed in 10- to 12-month-old SAMP8 could be significantly attenuated by two high affinity σ agonists, JO-1784 (igmesine) and PRE-084, administered subcutaneously in the mg/kg range, on several tests of mnemonic capacities (Maurice et al., 1996). δ-OR binding is decreased in the amygdala and ventral putamen, and μ-OR binding is decreased in the hippocampus and subiculum of post-mortem brain samples from patients with AD. In addition, there is an elevated hippocampal level of enkephalin, the ligand for these receptors, in the human AD brain (for a review see Thathiah & De Strooper, 2011).

### 4.11 Orexin receptors

The orexins (orexin-A and orexin-B, also known as hypocretin-1 and hypocretin-2) are neuropeptides derived from a single precursor expressed in a few thousand neurons restricted to the posterior lateral and medial hypothalamus. Orexin-A is the selective endogenous agonist for OX1 receptor, which is coupled to the activation of phospholipase C via a $G_s/G_q$ protein (Zhu et al., 2003). Inactivation of the receptor has been shown to cause impaired spatial learning in anaesthetized rats (Akbari et al., 2006). The effect of post-

training intracerebroventricular administration of Orexin-A on retention in active and passive avoidance has been reported in young (4 months) and old (12 months) SAMP8 mice (Jaeger et al., 2002). Orexin-A improved retention in young and old SAMP8 mice. However, no data about orexin receptor levels have been reported to date. Of interest is the observation that Aβ produced by neurons and secreted into the brain interstitial fluid is modulated by orexin in transgenic (Tg2576) mice, which express a mutated form of human amyloid precursor protein (APP) (Kang et al., 2009). Hypocretin-1 levels were normal in AD, although fragmentation of daytime wake was elevated in those with low hypocretin-1 levels. Thus, lower hypocretin-1 levels may be permissive for, or a consequence of, increased wake fragmentation in AD (Friedman et al., 2007).

### 4.12 Parathyroid hormone receptors

Parathyroid hormone (PTH), parathyroid hormone-related protein (PTHrP), and tuberoinfundibular peptide of thirty-nine residues (TIP39) are endogenous ligands for the parathyroid hormone 1 and 2 receptors (PTH1, PTH2). PTH is a classic endocrine hormone essential for mineral homeostasis (Potts et al., 1995). With advancing age (3, 6, 9, 12, and 15 months), the plasma PTH level increased progressively in female SAMP8 (Chen et al., 2007). In male SAMP8 and SAMR1 mice blood samples were collected monthly from 3 to 12 months of age. With advancing age, the plasma PTH level increased progressively in both SAMR1 and SAMP8 (Chen at al., 2004). The increase in serum PTH level has also been reported in older people and has been associated with impaired cognitive function, although the predictive value of PTH for cognitive decline has not yet been fully investigated (Bjorkman et al., 2010).

### 4.13 Somatostatin receptors

The somatostatin receptor family consists of 5 subtypes (sst1-5) each differentially distributed throughout the CNS and periphery. These receptors bind with high affinity to the endogenous polypeptides somatostatin-14, somatostatin-28 and cortistatin, as well as many other synthetic ligands. Receptor activation stimulates multiple intracellular signaling mechanisms giving rise to many tissue functions such as inhibition of growth hormone (GH) release and modulation of neuronal activity (Moller et al., 2003). The protein expression profile changes in the frontal cortex brain of SAMP8 model by means of microarray and RT-PCR techniques has recently been analyzed (Chen at al., 2010). In this study, somatostatin gene expression was lower in 12-month-old as compared with 4-month-old SAMP8 mice. Interestingly, the administration of NNC 26-9100, an sst4 receptor agonist, to 12-month-old SAMP8 mice did not modulate the expression of the sst4 receptor and APP when compared to vehicle control mice after 28 days of chronic NNC treatment, but it did enhance learning and memory (Sandoval et al., 2011). In addition, the expression of somatostatin did not change during aging in the pancreas of male SAMP8 and SAMR1 mice as no significant differences were observed between old (10 month) and young (2 month) mice. However, the expression of somatostatin was higher in the pancreas of young SAMP8 mice as compared with young SAMR1 mice (Cuesta et al., 2011). The expression of sst2 and sst4 is reduced in the cortex of human patients with AD (Kumar, 2005). Moreover, somatostatin levels are also reduced in the CSF, cortex and hippocampus of patients with AD (Thathiah & De Strooper, 2011).

### 4.14 Thyrotropin-releasing hormone receptors

Thyrotropin-releasing hormone (TRH) is a tripeptide (pyroGlu-His-Proamide) that is synthesized in the hypothalamus and released into the hypothalamic-pituitary portal circulation to act on the pituitary. TRH is produced in many other tissues, especially within the nervous system, where it appears to act as a neurotransmitter/neuromodulator. TRH receptors ($TRH_1$, $TRH_2$) belong to the Class A GPCR family. $TRH_2$ receptor has not been found in humans (Straub et al., 1990). Subcutaneous injection of a sustained release formulation of thyrotropin-releasing hormone (TRH-SR) produced a sustained increase in immunoreactive plasma TRH levels up to about 4 weeks after dosing in 8-month-old SAMP8. Furthermore, TRH-SR significantly improved the impairment of water maze learning in SAMP8 mice (Miyamoto et al., 1994a). TRH-SR also ameliorates impairments of learning behavior and the emotional disorder in SAMP8. In addition, SAMP8 shows an age-dependent abnormality of circadian rhythms of spontaneous motor activity (SMA) and ingestive behavior compared with the SAMR1 control, with diurnal SMA and water intake in SAMP8 being higher than in SAMR1 (Miyamoto, 1994b). These results suggest a reduced TRH level in SAMP8 mice. TRH concentration was decreased in the AD hippocampus compared to normal elderly controls (Luo et al., 2002). However, no significant differences from non-neuropsychiatric controls were noted on TRH receptor levels within the hippocampus in AD; just a slight alteration was noted in the cortical amygdala in AD (Lexow et al., 1994).

### 4.15 PACAP receptors

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are members of a superfamily of structurally related peptide hormones that includes glucagon, glucagon-like peptide, secretin and growth hormone-releasing hormone (GHRH). At least three receptors for PACAP exist in mammals, two of which are also high-affinity receptors for VIP (Harmar et al., 1998). In SAMP88 mice, PACAP is transported across the BBB in almost all regions of the brain, with the highest rates of transport found in the hypothalamus and hippocampus. This transport is lower in aged (12-month-old) than in young (2-month-old) mice. Of the differences between young and aged SAMP8 mice, the most interesting is the loss of the ability to transport PACAP into the olfactory bulb (Nonaka et al., 2002). Several studies have found abnormalities of the olfactory bulb of both SAMP8 mice (Ueno et al., 1998) and patients with Alzheimer's disease (Kovacs et al., 2001).

### 4.16 Metabotropic glutamate receptors

Glutamate is the main excitatory neurotransmitter in the central nervous system which has been implicated in several physiological and pathological processes (Conn & Pin, 1997). The different actions of glutamate are mediated through glutamate receptor binding, which has been classified into ionotropic and metabotropic. Metabotropic glutamate receptors (mGluRs) are coupled, through G proteins, to different effector systems, including phospholipase C (PLC) and adenylyl cyclase (AC). They have been classified into three groups on the basis of their pharmacological profile, molecular properties, and transduction mechanisms. Group I receptors ($mGlu_1$, $mGlu_5$) are coupled to PLC activation, through $G_{q/11}$ proteins, whereas groups II and III are coupled to AC inhibition, through $G_{i/o}$ proteins

(Conn & Pin, 1997). Preliminary results by our group suggest that mGluRs are impaired in SAMP8 mice (unpublished data). Interestingly, the mGluR/phospholipase C signaling pathway is impaired in the cerebral cortex in AD patients. Moreover, a decrease in mGluR specific binding correlates well with stage of AD-related changes (Albasanz et al., 2005). The efficacy of glutamatergic neurotransmission also relies on glutamate uptake and release from vesicles (Ishikawa et al., 2002; Wojcik et al., 2004). Vesicular glutamate transporters (VGLUTs), which include VGLUT1, VGLUT2 and VGLUT3, are responsible for the uploading of L-glutamate into synaptic vesicles and they are specifically required for exocytotic release (Reimer & Edwards, 2004). For glutamatergic synapses, a single functional vesicular glutamate transporter is both necessary and sufficient to fill a synaptic vesicle. However, elevated VGLUT expression increases the quantal size of a vesicle, and vesicles without VGLUT are empty (Daniels et al., 2006). Therefore, the expression level of VGLUTs determines the amount of glutamate that is loaded into vesicles and released, and thereby regulates the efficacy of neurotransmission (Ishikawa et al., 2002; Wojcik et al., 2004). Protein expression of VGLUT1, VGLUT2, VGLUT3 and synaptophysin (Syp), a marker of synapse (Kashani et al., 2008), tends to decrease in the hippocampus, and was significantly decreased in an age-dependent manner in the cerebral cortex of SAMP8 with age-related deterioration of learning and memory (Cheng et al., 2011), which could indicate that the glutamatergic synaptic transmission was weakened in the brain of aging SAMP8. Consistent with a dysfunction in the recycling of glutamate, there is a selective loss of vesicular glutamate transport in synaptic vesicles isolated from cerebral cortex synaptosomes from AD (Westphalen et al., 2003).

| GPCR | AD | SAMP8 |
|---|---|---|
| 5-Hydroxytryptamine receptors | ↓ 5-$HT_{2A}$, 5-$HT_4$, 5-$HT_6$ | ↓ serotonergic activity (5-$HT_1$, 5-$HT_2$) |
| | ↓ 5-HT level | ↓ 5-HT level |
| Acetylcholine receptors (muscarinic) | ↓ hippocampal $M_1$ | ↓ hippocampal $M_1$ |
| | ↑ frontal cortical $M_1$ | ↑ cortical $M_1$ |
| | ↓ ACh release | ↓ ACh release |
| Adenosine receptors | ↑ frontal cortical $A_1$, $A_{2A}$ | ↓ $A_1$, ↑ $A_{2A}$ (whole brain) |
| Adrenoceptors | ↑ prefrontal cortical and hippocampal $\beta_2$AR | no apparent change |
| Calcitonin receptors | ↑ amylin receptor (TgCRND8 mice) | ↓ plasma calcitonin |
| Chemokine receptors | ↑ CCL2 | ↑ hippocampal Ccl19, Ccl27 expression, ↑ CCL2 |
| Dopamine receptors | ↓ frontal cortical $D_1$, $D_3$, $D_4$, $D_2$<br>↑ frontal cortical $D_5$<br>↓ temporal lobe DR | n.d. |

| GPCR | AD | SAMP8 |
|---|---|---|
| | ↓ DA level | ↓ DA level |
| $GABA_B$ receptors | ↓ hippocampal and cortical $GABA_B$ | n.d. |
| | ↓ GABA level in CSF | n.d. |
| Melatonin receptors | ↓ $MT_2$ | = $MT_1$, $MT_2$ mRNA expression |
| | ↓ melatonin level in CSF | n.d. |
| Opioid receptors | ↓ δ-OR amygdala and putamen<br>↓ μ-OR hippocampus and subiculum | ↓ cortical σ site<br>= hippocampal σ site |
| | ↑ hippocampal enkephalin | n.d. |
| Orexin receptors | = Hypocretin-1 level | n.d. |
| Parathyroid hormone receptors | = PTH level (but ↑ PTH with aging) | ↑ PTH level with aging |
| Somatostatin receptors | ↓ sst2, sst4 | n.d. |
| | ↓ somatostatin level | ↓ somatostatin level |
| Thyrotropin-releasing hormone receptors | = hippocampal TRH receptors<br>↓ cortical amygdala TRH receptor | n.d. |
| | ↓ TRH level | ↓ TRH level |
| PACAP receptors | ↓ PACAP expression | Altered transport through BBB |
| Metabotropic glutamate receptor | ↓ mGluR in frontal cortex | not published |
| | ↓ VGLUT | ↓ VGLUT |

Table 3. G protein-coupled receptors assessed in Alzheimer's disease (AD) and senescence-accelerated mouse P8 strain (SAMP8). n.d.: not determined. GPCRs that have been not investigated in SAMP8, or at least not found in literature, are the following: Anaphylatoxin, Angiotensin, Apelin, Bile acid, Bombesin, Bradykinin, Calcium-sensing, Cannabinoid, Cholecystokinin, Corticotropin-releasing factor, Endothelin, Estrogen (G protein-coupled) Formylpeptide, Free fatty acid, Frizzled, Galanin, Ghrelin, Glucagon receptor family, Glycoprotein hormone, Gonadotrophin-releasing hormone, Histamine, Hydroxycarboxylic acid, Kisspeptin, Leukotriene, Lysophospholipid, Melanin-concentrating hormone, Melanocortin, Motilin, Neuromedin U, Neuropeptide FF/neuropeptide AF, Neuropeptide S receptor, Neuropeptide W/neuropeptide B, Neuropeptide Y, Neurotensin, P2Y, Peptide P518, Platelet-activating factor, Prokineticin, Prolactin-releasing peptide, Prostanoid, Protease-activated, Relaxin family peptide, Tachykinin, Trace amine, Urotensin and Vasopressin and oxytocin receptors.

## 5. Conclusion

Senescence-accelerated mouse P8 (SAMP8) has many features that are known to occur early in the pathogenesis of AD such as increased oxidative stress, amyloid-β alterations, and tau phosphorylation. The neurochemical changes reported to date in SAMP8 mice may help in the study of AD pathogenesis as many of them are similar to what is found in AD (see Table 3). However, there is still a lot of work to be done in the analysis of the different receptor-mediated signaling pathways and their modulation in this animal model.

## 6. Acknowledgment

We are very grateful to Dr. Antoni Camins and Dr. Mercé Pallas for their contributions to the study of SAMP8 mice and the Fundació La Marató de TV3 (092330, 090331) for funding. The experimental work described in the present chapter about adenosine receptor modulation was funded in part by the European Union through the Marie-Curie Research Training Network PRAIRIES (Contract MRTN-CT-2006-035810), by the Consejería de Educación y Ciencia (PCI08-0125), the Consejería de Sanidad (PI-2007/50 and G-2007-C/13) of the Junta de Comunidades de Castilla-La Mancha, the Ministerio de Ciencia e Innovación (BFU2008-00138 and SAF2006-13092) and the Instituto de Salud Carlos III (PI080400). We thank Tom Yohannan for editorial assistance.

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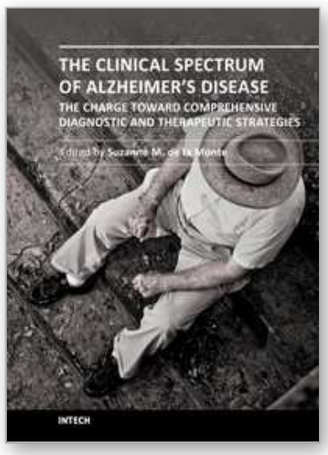


**The Clinical Spectrum of Alzheimer's Disease -The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies**

Edited by Dr. Suzanne De La Monte

ISBN 978-953-307-993-6
Hard cover, 362 pages
**Publisher** InTech
**Published online** 06, September, 2011
**Published in print edition** September, 2011

The Clinical Spectrum of Alzheimer's Disease: The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies is highly informative and current. Acknowledged experts in the field critically review both standard and under-appreciated clinical, behavioral, epidemiological, genetic, and neuroimaging attributes of Alzheimer's disease. The collection covers diverse topics of interest to clinicians and researchers alike. Experienced professionals and newcomers to the field will benefit from the read. The strengths and weaknesses of current clinical, non-invasive, neuro-imaging, and biomarker diagnostic approaches are explained. The perspectives give fresh insights into the process of neurodegeneration. Readers will be enlightened by the evidence that the neural circuits damaged by neurodegeneration are much broader than conventionally taught, suggesting that Alzheimer's could be detected at earlier stages of disease by utilizing multi-pronged diagnostic approaches. This book inspires renewed hope that more effective treatments could be developed based upon the expanding list of potential therapeutic targets.

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José Luis Albasanz, Carlos Alberto Castillo, Marta Barrachina, Isidre Ferrer and Mairena Martín (2011). Modulation of Signal Transduction Pathways in Senescence-Accelerated Mice P8 Strain: A Useful Tool for Alzheimer's Disease Research, The Clinical Spectrum of Alzheimer's Disease -The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies, Dr. Suzanne De La Monte (Ed.), ISBN: 978-953-307-993-6, InTech, Available from: http://www.intechopen.com/books/the-clinical-spectrum-of-alzheimer-s-disease-the-charge-toward-comprehensive-diagnostic-and-therapeutic-strategies/modulation-of-signal-transduction-pathways-in-senescence-accelerated-mice-p8-strain-a-useful-tool-fo

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