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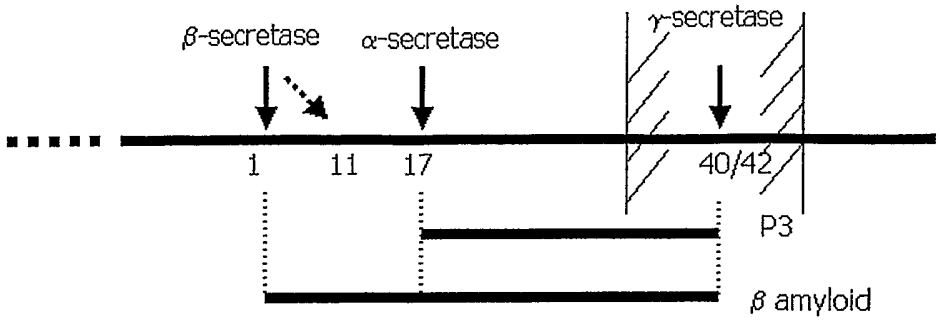
(54) Title: NOVEL COMPOUNDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE AND METHODS FOR IDENTIFYING SAME



(57) Abstract: The present invention relates to methods of identifying compounds that influence the amyloid-beta precursor protein processing in a cell, and/or that influence the activity or levels of proteins involved in the processing of the amyloid beta precursor protein. It furthermore relates to the compounds that may be identified using the methods of the invention and to use of said compounds in the treatment of neurological disorders such as Alzheimer's disease. More specifically, it relates to compounds that target the melanocortin 3 and 4 receptors (MC3R and MC4R).

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TITLE

Novel compounds for the treatment of Alzheimer's disease and methods for identifying same.

FIELD OF THE INVENTION

The invention relates to novel compounds and to methods for identifying such compounds. The compounds typically target the melanocortin 3 and 4 receptor (MC3R and MC4R), and are useful in the treatment of neurological disorders such as Alzheimer's disease. More specifically, the invention relates to compounds that influence the processing of the amyloid-beta precursor protein.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a neurological disorder that is clinically characterized by the progressive loss of intellectual capacities: initial loss of memory, and later on by disorientation, impairment of judgment and reasoning, commonly referred to as cognitive impairment, progressing to full dementia. The patients finally fall into a severely debilitated, immobile state between 4 and 12 years after onset of the disease. Worldwide, about 20 million people suffer from AD. The pathological hallmarks of the disease are the presence of extracellular amyloid plaques and intracellular tau tangles in the brain, which are associated with neuronal degeneration (Ritchie and Lovestone, 2002).

A small fraction of AD cases are caused by autosomal dominant mutations in the genes encoding presenilin 1 and 2 (PS1; PS2) and the amyloid-beta precursor protein (APP). It has been shown that mutations in APP, PS1 and/or PS2 alter the amyloid-beta precursor protein metabolism (processing) such that more of the

insoluble, pathogenic amyloid beta 1-42 is produced in the brain. Following secretion, these amyloid beta 1-42 peptides form amyloid fibrils more readily than the amyloid beta 1-40 peptides, the latter being predominantly produced in healthy people. The insoluble amyloid fibrils resulting from the amyloid beta 1-42 peptides are subsequently deposited in the amyloid plaques, resulting in the onset and/or the progression of the disease.

The amyloid beta peptides are generated from the membrane anchored APP after cleavage by beta secretase and gamma secretase at position 1 and 42, respectively (Figure 1, Annaert and De Strooper, 2002). The gamma secretase can also cleave at position 40. In addition, it was found that an increased activity of beta secretase might result in a shift of the cleavage at position 1 to position 11. Cleavage of amyloid-beta precursor protein by alpha secretase activity and gamma secretase activity at position 17 and 40 or 42 generates the non-pathological p3 peptide. The beta secretase protein was identified as the membrane anchored aspartyl protease BACE, while gamma secretase is a protein complex comprising presenilin 1 (PS1) or presenilin 2 (PS2), nicastrin, Anterior Pharynx Defective 1 (APH1) and Presenilin Enhancer 2 (PEN2). Of these proteins, the presenilins are thought to constitute the catalytic activity of the gamma secretase, while the other components play a role in the maturation and localization of the complex. The identity of the alpha secretase is still illustrious, although some results point towards the proteases ADAM 10 and TACE, which could have redundant functions.

It has been shown that injection of amyloid beta fibrils in the brains of P301L tau transgenic mice

enhances the formation of neurofibrillary tangles, placing the amyloid beta peptide upstream of the neurotoxic cascade (Gotz et al. 2001). Although no mutations in PS1, PS2 and amyloid-beta precursor protein have been identified in late onset AD patients, the pathological hallmarks are highly similar to the early onset AD patients. Therefore, it is generally accepted that aberrant increased amyloid peptide levels in the brains of late onset AD patients are also the cause of the disease. These increased levels of amyloid beta peptide could originate progressively with age from disturbed amyloid-beta precursor protein processing (e.g. high cholesterol levels enhance amyloid beta peptide production) or from decreased catabolism of the peptide.

Because the cholinergic neurons are the first neurons to degenerate during AD, levels of the neurotransmitter acetylcholine decrease, which results in the progressive loss of memory. Therefore, the major current AD therapies are focused on the inhibition of the acetylcholinesterase enzyme, leading to an increased concentration of the acetylcholine. However, this therapy does not halt the progression of the disease. Due to the fact that the socio-economical impact of AD is worldwide considered very significant, the need for an effective therapy is extremely high.

Therapies aimed at decreasing the levels of amyloid beta peptides in the brain, are investigated in the field. Most of these therapies are focused on the perturbed amyloid-beta precursor protein processing (metabolism) and target directly beta- or gamma secretase activity. However, targeting these proteins has not yielded any new drugs to date, because of the difficulty to find specific drugs and of suspected serious side effects.

Thus, the identification of alternative drug targets within the amyloid-beta precursor protein-processing pathway is of great importance. This would allow a direct interference with the production of the pathological amyloid beta 1-42 peptide, which preferably should block the neurotoxic cascade induced by the latter.

In 1993, Gantz et al. (1993) cloned the human melanocortin 4 receptor (MC4R). Together with MC1, MC2, MC3 and MC5, the MC4 receptor forms the family of the melanocortin receptors. All the melanocortin receptor subtypes are coded on a single exon. These receptors all belong to the class of the G protein-coupled receptors. All melanocortin receptors couple to the Gs class of G-proteins and elevate intracellular cAMP levels. In contrast to the MC1, -2 and -5 receptors the MC3 and -4 receptors are exclusively expressed in the Central Nervous System (CNS), whereas the other 3 receptors are expressed only in the periphery. The MC4R is widely expressed in the CNS, although concentrated expression is seen in the hypothalamus and brainstem. Disruption or antagonism of this receptor causes an obesity syndrome characterized by moderate hyperphagia and obesity, mild hyperinsulinemia, increased linear growth, but otherwise relatively normal neuroendocrine functions. This receptor is clearly important in coordinating energy expenditure with energy intake in response to both short term and long-term peripheral metabolic signals; haploinsufficiency of the MC4R is responsible for at least 5% of severe human obesity. The melanocortin peptides are processed from three different regions of POMC, each of which contains a conserved sequence, -His-Phe-Arg-Trp-, that serves as the pharmacophore for melanocortin receptor activation. Peptides derived from

the amino terminus of POMC are called ã-MSH (ã-Melanocyte stimulating hormone) peptides. Adrenocorticotropic hormone (ACTH) and á-MSH are overlapping peptides cleaved from the middle portion and consist of amino acids 1-39 and 1-13 of this middle region, respectively. â-MSH, â-LPH (â-lipotropin) and ã-LPH all derive from the carboxy-terminal portion of the POMC precursor. A curious feature of the melanocortin receptors is the existence of a family of endogenous antagonists, the agouti proteins. Agouti is a 131 amino acid peptide made by dermal follicular cells that acts as a specific high affinity antagonist of the MC1R. Agouti related protein (AGRP), a homologue made in the arcuate nucleus of the hypothalamus and the adrenal gland, is an antagonist of the central MC3R and MC4R (Ollmann et al. 1997).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic outline of APP processing. The membrane anchored amyloid precursor protein (APP) is processed by two pathways: the amyloidogenic and non amyloidogenic pathway. In the latter pathway, APP is cleaved first by alpha secretase and then by gamma secretase, yielding the p3 peptides (17-40 or 17-42). The amyloidogenic pathway generates the pathogenic amyloid beta peptides (A beta) after cleavage by beta- and gamma-secretase respectively. The numbers depicted are the positions of the amino acids comprising the A beta sequences.

Figure 2. Evaluation of the APP processing assay. Positive (PS1G384L; PS1L392V and BACE1) and negative (eGFP, LacZ and empty) control viruses are infected in Hek293APPwt at random MOI, mimicking a screening. A and B: Transduction is performed respectively with 1 and 0.2

µl of virus and amyloid beta 1-42 levels are performed. Data are represented as relative light units and correlate to pM of amyloid beta 1-42.

Figure 3. Involvement of MC4R in APP processing. HEK293 APPwt cells are transduced with Ad5/MC4R and with negative control viruses (Ad5/empty, Ad5/eGFP and Ad5/luciferase) at different MOIs (2, 10, 50, 250). Resulting amyloid beta 1-42, 1-40, peptides were measured with the appropriate ELISA's. Data are represented in pM or as relative light units (rlu), which correlates to pM of amyloid beta.

Figure 4. Agonist antagonist experiment MC4R. HEK293 APPwt cells are transduced with Ad5/MC4R and with Ad5/Empty (negative control). 24 h after infections the medium is replaced with medium containing ligands in increasing concentrations. After 24 h amyloid beta peptide 1-42 is determined with a specific ELISA. Data are presented in pM amyloid beta secreted.

Figure 5. ClustalW protein sequence alignment of MC4R and MC3R.

SUMMARY OF THE INVENTION

The present invention relates to the association between melanocortin receptors and the production and processing of amyloid beta. More specifically, the invention provides novel compounds that interfere with the processing of the amyloid beta precursor protein processing through the targeting of certain melanocortin receptors, especially the MC4 receptor MC4R.

The present invention provides novel methods to identify compounds that change the amyloid-beta precursor

protein processing in cells. It also relates to methods to influence the processing of the amyloid-beta precursor protein by using said compounds. Methods are directed to decrease the expression of the G-protein coupled receptor MC4R. Decrease of expression of MC4R results in reduction of amyloid-beta peptide 1-42.

Different compounds that can be identified according to the methods provided by the present invention and used in methods for the treatment of diseases such as Alzheimer's disease are antisense RNA, Ribozymes, antisense oligodeoxynucleotides, small molecules and small interfering RNA. These compounds typically cause the decrease of expression of MC4R. Polypeptides and polynucleotides, and vectors comprising same, are also provided. The compounds can also be polyclonal or monoclonal antibodies that interact with the polypeptides and inhibit their activity. The compounds may also be 'nanobodies', the smallest functional fragment of naturally occurring single-domain antibodies (Cortez-Retamozo et al. 2004).

The compounds according to the present invention are useful as a medicament, preferably in the treatment of Alzheimer's disease. The present invention furthermore provides means and methods to change the processing, the activity and the levels of the amyloid-beta precursor protein, for instance by inhibiting the activity of the protein MC4R. Thus, compounds that inhibit the activity of MC4R are provided. Also provided are methods for diagnosis of pathological conditions involving cognitive impairment, such as AD. This invention also provides methods and compounds, as outlined above, in relation to MC3R.

DETAILED DESCRIPTION

This invention relates to the targeting with compounds of the melanocortin 3 and 4 receptors (MC3R and MC4R), which targeting typically results in a reduction of amyloid beta 1-42 levels in the conditioned medium of transduced cells. As disclosed herein, MC4R (and its related protein MC3R) are identified as putative drug targets for the (prophylactic) treatment and/or the diagnosis of Alzheimer's disease (AD).

Compounds that reduce or inhibit the activity of MC3R and/or MC4R have never been associated with treatment or prevention or amelioration of a pathological condition involving cognitive impairment, such as AD. The present invention clearly indicates that reduction of expression or reduction of activity of MC4R is related to the reduction of the amyloid-beta peptide level, which reduction is desired.

The present invention relates to a method of identifying a compound that modifies the amyloid-beta precursor protein processing (metabolism) in a cell, which method comprises providing a population of cells expressing a polypeptide having an amino acid sequences selected from the group consisting of SEQ ID NO: 3 or 4, or a functional fragment or derivative thereof; determining a first amyloid-beta precursor processing level in the cell; exposing the cell to a compound; determining a second amyloid-beta precursor processing level in the cell; and identifying the compound that modifies the amyloid-beta precursor processing in said cell. Preferably, the compound identified according to the methods of the present invention is selected from the group consisting of an antisense RNA molecule, a ribozyme (that cleaves the polyribonucleotide), an antisense

oligodeoxynucleotide (ODN), a small interfering RNA (siRNA, that is sufficiently homologous to a portion of the polyribonucleotide such that the siRNA is capable of inhibiting the polyribonucleotide that would otherwise cause the production of the polypeptide), a nucleic acid expressing the antisense RNA, an antibody, a polypeptide, a peptide, a lipid, a polynucleotide, or a vector carrying a polynucleotide or peptide according to the invention. Preferably, the invention relates to a method wherein the modification in the processing of the amyloid-beta precursor protein in a cell is a reduction of the level of amyloid-beta peptide 1-42, 1-40, 11-42 or 11-40, most preferably a reduction of the amyloid beta peptide 1-42. The present invention further relates to a method of identifying a compound that modifies the amyloid-beta precursor protein processing (metabolism) in a cell, which method comprises expressing a polypeptide having an amino acid sequences selected from the group consisting of SEQ ID NO: 3 or 4, or a functional fragment or derivative thereof; determining a first activity level of said polypeptide; exposing the cell to a compound; determining a second activity level of said polypeptide; and identifying the compound that influences the activity level. Preferably, the activity is measured through measuring the level of one or more second messengers of the polypeptide. Typically, the invention relates to a method wherein the second activity level is lower than the first activity level. More preferably, the second activity level is close to zero, meaning that the activity of the target protein has been lowered to levels that would be beneficial in the treatment and/or prevention of the disease that needs to be treated and/or prevented.

Although a reduction of the levels may differ and may be multifold, it is held here that a reduction of 30% in a patient (in vivo) is a preferred level. Thus the influence on the processing on the amyloid-beta precursor protein (APP) as used herein refers to a preferred reduction of said expression and/or activity which is more or less comparable to a reduction of 30% (or more) in vivo. It can however not be excluded that levels found in vitro do not perfectly correlate with levels found in vivo, such that a slightly reduced level in vitro may still result in a higher reduction in vivo when the compound is applied in a therapeutic setting. It is therefore preferred to have reduced in vitro levels of at least 10%, more preferably more than 30%, even more preferably more than 50% and most preferably a reduction between 50% and 100%, which would mean an almost complete disappearance of the amyloid-beta peptides 1-42, 1-40, 11-42 and/or 11-40.

The present invention relates to a method of identifying a compound that decreases the expression and/or activity of a polypeptide selected from SEQ ID NO: 3 or 4 in a cell, said method comprising the steps of incubating a cell; determining a first level of expression and/or activity of said polypeptide in said cell; incubating said cell with a compound; determining a second level of expression and/or activity in said cell of said polypeptide following or during the previous step; and identifying a compound that decreases the expression and/or activity of said polypeptide.

The present invention relates to a novel identified process, wherein inhibition or level reduction of the melanocortin 3 and 4 receptors (MC3R and MC4R) results in a decrease in the level of amyloid beta precursor protein processing. Over-expression or activation of the MC3R and

MC4R proteins increase the level of secreted amyloid beta 1-42, amyloid beta 11-42, amyloid beta 1-40, amyloid beta 1-x, where x ranges from 24-42, and amyloid beta Y-42, where y ranges from 1-17.

The amyloid beta peptides 1-42, 1-40, 1-39, 1-38, 1-37 are often seen in cerebral spinal fluid. Amongst other amyloid beta peptides, the amyloid beta peptide 1-42 was found in amyloid plaques. Thus, reducing the level of this amyloid beta peptide is beneficial for patients with cognitive impairment. The pharmacological inhibition of these targets results in a decrease of amyloid beta levels. A preferred polypeptide that is targeted by the compounds of the present invention is a G-protein coupled receptor (GPCR) and can typically be inhibited by small molecules. GPCRs, their general structure and the signal transduction pathways that involve GPCRs are well known in the art.

All GPCRs share a common architecture of 7 transmembrane domains, an extracellular N-terminus and an intracellular C-terminus. The major signal transduction cascades activated by GPCRs are initiated by the activation of heterotrimeric G-proteins, built from three different proteins; the G_α, G_β and G_γ subunits. The signal transduction cascade starts with the activation of the receptor by an agonist. Transformational changes in the receptor are then translated down to the G-protein. The G-protein dissociates into the G_α subunit and the $G_{\beta\gamma}$ subunit. Both subunits dissociate from the receptor and are both capable of initiating different cellular responses. Best known are the cellular effects that are initiated by the G_α subunit. It is for this reason that G-proteins are categorized by their G_α subunit. The G-proteins are divided into four groups: G_s , $G_{i/o}$, G_q and $G_{12/13}$. All G-proteins are capable of activating an

effector protein, which in turn results in changes in second messenger levels in the cell. The changes in second messenger level are the triggers that make the cell respond to the extracellular signal in a specific manner. The inventors of the present invention have made use of this: The activity of a GPCR can be measured by measuring the activity level of the second messenger.

The two most important second messengers in the cell are cAMP and Ca^{2+}, and are the preferred second messengers used in the methods according to the present invention for determining the activity level of the targeted polypeptide. The α-subunit of the G_s class of G-proteins is able to activate adenylyl cyclase, resulting in an increased turnover from ATP to cAMP. The α-subunit of $G_{i/o}$ G-proteins does exactly the opposite and inhibits adenylyl cyclase activity resulting in a decrease of cellular cAMP levels. Together, these two classes of G-proteins regulate the second messenger cAMP. Ca^{2+} is regulated by the α-subunit of the G_q class of G-proteins. Through the activation of phospholipase C phosphatidylinositol 4,5-bisphosphate (PIP2) from the cell membrane are hydrolyzed to inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both these molecules act as second messengers. Inositol 1,4,5-trisphosphate binds specific receptors in the endoplasmatic reticulum, resulting in the opening of Ca^{2+} channels and release of Ca^{2+} in the cytoplasm. Determining the level of the second messenger is hence found useful in determining the biological activity of the GPCR. The second messenger levels can be measured by several techniques known in the art, either directly by ELISA or radioactive technologies or indirectly by reporter gene analysis.

A host cell expressing a polypeptide of the present invention can be a cell with endogenous expression of the polypeptide or a cell overexpressing the polypeptide e.g. by transduction. When the endogenous expression of the polypeptide of the present invention is not sufficient for a first activity level of the second measure that can easily be measured, overexpression of the polypeptide can be applied, or an agonist of the polypeptide can be added. Overexpression has the advantage that the first activity level of the second messenger is significantly higher than the activity level by endogenous expression and differences can be measured more easily.

In one embodiment of the present invention, the method according to the present invention comprises the step of contacting the host cell with an agonist for the polypeptide before determining the first activity level. The addition of an agonist further stimulates the polypeptide of the present invention, thereby further increasing the activity level of the second messenger if the endogenous levels do not allow proper measurements.

According to one embodiment, the MC4R and MC3R proteins are activated with a ligand, here also called an agonist of the GPCRs. A method to identify a compound hence may further include contacting the host cell with an agonist of the polypeptide before measuring the level of one or more second messengers of the polypeptide. Several agonists are known in the art, and include amongst others, alpha-MSH, beta-MSH, gamma-MSH, beta-LPH, gamma-LPH, ACTH, Ro 27-3225 and MTII. Preferred agonists are Ro273225, MTII and alpha-MSH.

The amyloid-beta precursor protein is processed into several different amyloid beta peptides species. According to the methods provided by the present invention, compounds are identified that change the APP

processing and reduce the level of secreted pathological amyloid beta peptides. Levels of amyloid beta peptides can be measured with specific ELISA's using an antibody specifically recognizing the amyloid beta peptide species 1-42 (see e.g. Example 1). Levels of amyloid beta peptides can also be measured by Mass spectrometry analysis. A particularly preferred embodiment of the present invention relates to a method wherein the polypeptide is MC4R, as defined by SEQ ID NO:3.

A further embodiment of the present invention relates to methods for identifying a compound that influences the amyloid-beta precursor protein processing in a cell, wherein the activity level of the polypeptide is measured by determining the level of one or more second messengers of the polypeptide, and wherein the level of the one or second messenger is determined with a reporter comprising a promoter that is responsive to the second messenger.

The reporter typically comprises a gene under the control of a promoter that responds to the cellular level of a second messenger. Preferred second messengers are Cyclic AMP or $Ca2^{+}$. The reporter gene encodes a gene product that can easily be measured. The reporter gene can either be stably transfected (integrated into the host cell genome) or be present in the cell outside the genome.

The reporter gene is preferably selected from the group consisting of alkaline phosphatase, Green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), destabilized green fluorescent protein (dGFP), luciferase, and β-galactosidase. Other reporter genes may be applied.

Preferably the promoter controlling the reporter construct is a cyclic AMP-responsive promoter, an NF-KB responsive promoter, or a NF-AT responsive promoter. The cyclic-AMP responsive promoter is responsive to the cyclic-AMP levels in the cell. The NF-AT responsive promoter is sensitive to cytoplasmic Ca^{2+}-levels in the cell. The NF-KB responsive promoter is sensitive for activated NF-κB levels in the cell.

Preferably the reporter is luciferase or β-galactosidase. Luciferase and β-galactosidase are easily available and have a large dynamic range for measuring. In addition, luciferase and β-galactosidase are less expensive which is favorable especially when performing the method of the present invention in a high throughput format.

The present invention further relates to methods for identifying compounds that influences the amyloid-beta precursor protein processing (metabolism) in a cell, which methods comprise the steps of contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3 or 4, or a functional derivative or fragment thereof; determining the binding affinity of the compound to the polypeptide; contacting a population of cells expressing the polypeptide with the compound that exhibits a binding affinity of at most 10 micromolar; and identifying the compound that influences the amyloid-beta precursor protein processing in the cells. Preferably, the levels of amyloid-beta are decreased. The decrease of amyloid-beta precursor protein is typically measured by determining the levels of one or more second messenger molecules. More in particular, the method relates to a method identifying a compound that influences the

amyloid-beta precursor protein processing in a cell, wherein said modification of amyloid-beta precursor protein processing in a cell results in a reduction of the level of amyloid-beta peptide 1-42, 1-40, 11-42 or 11-40, most preferably the 1-42 peptide.

The binding affinity of the compound with the polypeptide or polynucleotide can be measured by methods generally known in the art, such as using surface plasmon resonance biosensors (Biacore), by saturation binding analysis with a labeled compound (e.g. Scatchard and Lindmo analysis), by differential UV spectrophotometer, fluorescence polarisation assay, Fluorometric Imaging Plate Reader (FLIPR®) system, Fluorescence resonance energy transfer, and Bioluminescence resonance energy transfer. The binding affinity of compounds can also be expressed in dissociation constant (Kd) or as IC50 or EC50. The IC50 represents the concentration of a compound that is required for 50% inhibition of binding of another ligand to the polypetide. The EC50 represents the concentration required for obtaining 50% of the maximum effect in any assay that measures receptor function. The dissociation constant, Kd, is a measure of how well a ligand binds to the polypeptide, it is equivalent to the ligand concentration required to saturate exactly half of the binding-sites on the polypeptide. Compounds with a high affinity binding have low Kd, IC50 and EC50 values, i.e. in the range of 100 nM to 1 pM; a moderate to low affinity binding relates to a high Kd, IC50 and EC50 values, i.e. in the micromolar range.

For high-throughput purposes, libraries of compounds can be used such as peptide libraries (e.g. LOPAP™, Sigma Aldrich), lipid libraries (BioMol), synthetic compound libraries (e.g. LOPAC™, Sigma Aldrich) or natural compound libraries (Specs, TimTec).

One preferable type of compound that can be identified by the methods of the present invention is a low molecular weight compound. Low molecular weight compounds, i.e. with a molecular weight of approximately 500 Dalton or less, are likely to have good absorption and permeation in biological systems and are consequently likely to be successful drug candidates (Lipinski et al. 1997).

According to another embodiment the compounds are peptides. Many GPCRs have a peptide as an antagonist. Peptides can be excellent drug candidates and there are multiple examples of commercially valuable peptides such as fertility hormones and platelet aggregation inhibitors.

According to another embodiment the compounds are natural compounds. Natural compounds are compounds that have been extracted from e.g. plants, soil or tissues, or compounds or that may be synthesized on the basis of a natural occurring molecule. Using natural compounds in screens may have the advantage that one is able to screen more diverse kinds of molecules.

According to another embodiment the compounds are lipids. GPCRs listed in table 1 (SEQ ID NO:1-4) can have lipids as antagonists. Using lipids as candidate compounds can increase the chance of finding a specific antagonist or inverse agonist for the polypeptides of the present invention.

According to another embodiment, the compound is an antibody. The generation of specific antibodies against target proteins and/or other cellular factors is well known in the art.

According to another embodiment, the compounds are low molecular weight compounds selected from the

compounds as described in US patent 6,699,873 or 6,693,165. It is preferred that the compounds are able to pass the blood-brain barrier. Small molecule compounds that may be identified by using the present invention preferably act as antagonists of MC4R. The inventors of the present invention have identified several small molecule compounds and cyclic peptides that act as antagonists of MC4R, and that may be very suitable for changing the amyloid-beta precursor protein processing in a cell.

In another aspect of the present invention, the compound is an expression inhibitory agent inhibiting the expression and/or translation of a nucleotide sequence encoding a polypeptide selected from SEQ ID No: 3 or 4. Expression levels and/or translation levels of the proteins may be determined using general methods known in the art. Such methods include mRNA analysis by Northern blots, Reverse transcriptase PCR and Real-time PCR, amongst others. Other useful methods are protein analysis by Western blots or Elisa. One type of expression-inhibitory agent concerns a nucleic acid that is antisense to a nucleic acid comprising SEQ ID NO: 1 or 2. For example, an antisense nucleic acid (e.g. DNA) may be introduced into cells *in vitro*, or administered to a subject *in vivo*, as gene therapy to inhibit cellular expression of nucleic acids comprising SEQ ID NO: 1 or 2.

Antisense oligonucleotides preferably comprise a sequence containing from about 17 to about 100 nucleotides and more preferably the antisense oligonucleotides comprise from about 18 to about 30 nucleotides. Antisense nucleic acids may be prepared by expression of all or part of a sequence selected from the group consisting of SEQ ID NO: 1 or 2, in the opposite orientation.

Antisense oligonucleotides can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linkages, modified nucleic acid bases and/or modified sugars and the like.

The antisense oligonucleotides can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide. Such moieties or conjugates include lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), or palmityl moieties.

Another type of expression-inhibitory agent as a compound relates to a nucleic acid that is able to catalyze cleavage of RNA molecules. The expression "ribozymes" relates to catalytic RNA molecules capable of cleaving other RNA molecules at phosphodiester bonds in a manner specific to the sequence. The hydrolysis of the target sequence to be cleaved is initiated by the formation of a catalytically active complex consisting of ribozyme and substrate RNA. All ribozymes capable of cleaving phosphodiester bonds *in trans*, that is to say intramolecularly, are suitable for the purposes of the invention. Apart from ribonuclease P the known naturally occurring ribozymes (hammerhead ribozyme, hairpin ribozyme, hepatitis delta virus ribozyme, Neurospora mitochondrial VS ribozyme, group I and group II introns) are catalysts, which cleave or splice themselves and which act in cis (intramolecularly).

A preferred method of identifying compounds according to the present invention relates to the identification of small interfering RNAs (siRNAs). siRNAs mediate the post-transcriptional process of gene

silencing by double stranded RNA (dsRNA) that is homologous in sequence to the silenced RNA. siRNAs can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linkages, modified nucleic acid bases and/or modified sugars and the like. siRNAs can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide. Such moieties or conjugates include lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), or palmityl moieties.

One embodiment of the present invention relates to a method wherein the siRNA comprises a sense strand of 17-23 nucleotides homologous to a 17-23 nucleotide long nucleotide sequence selected from the group consisting of SEQ ID NO: 1 or 2, and an antisense strand of 17-23 nucleotides complementary to the sense strand. All nucleotides in the sense and antisense strand base pair, or alternatively there may be mismatches between the sense and antisense strand. Preferably the siRNA further comprises a loop region connecting the sense and the antisense strand.

A self-complementing single stranded siRNA molecule polynucleotide according to the present invention comprises a sense portion and an antisense portion connected by a loop region. Preferably, the second sequence is 4-30 nucleotides long, more preferably 5-15 nucleotides long and most preferably 8 nucleotides long. In a most preferred embodiment the linker sequence is GTTTGCTATAAC (SEQ ID NO: 171).

Self-complementary single stranded siRNAs form hairpin loops and are more stable than ordinary dsRNA. In addition, they are more easily produced from vectors. It is therefore preferred that the nucleotide comprising the siRNA present within a vector, which is preferably an adenoviral, retroviral, adeno-associated viral (AAV), lentiviral, herpes simplex viral (HSV), an alphaviral or a sendai viral vector.

Thus, the expression inhibitory agent may be an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 5 to 170. Nucleotide sequences of the siRNAs are generally selected according to siRNA designing rules known in the art that give an improved reduction of the target sequences compared to nucleotide sequences that do not comply with these siRNA designing rules.

A further aspect of the invention relates to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 5-170, or a nucleotide sequence complementary thereto, or a functional derivative or fragment thereof, or a pharmaceutical acceptable salt thereof for use as a medicament for the treatment of a pathological condition involving cognitive impairment or a susceptibility to the condition. Such pathological condition is preferably Alzheimer's disease.

In another embodiment, a polynucleotide according to the invention is modified to confirm resistance to nucleolytic degradation or to enhance the activity, cellular distribution, or cellular uptake. Such modification may consist of modified internucleoside linkages, modified nucleic acid bases, modified sugars,

and/or chemically linking the oligonucleotide to one or more moieties or conjugates.

Vectors that may be applied according to the present invention and that comprise nucleic acids according to any one of SEQ ID NO: 1, 2, 5-170 may also be used as a medicament, as discussed intra. The nucleotide sequence in the vector may be an siRNA, an antisense RNA, a ribozyme, or an antisense oligodeoxynucleotide according to the invention.

A further aspect of the invention relates to the use of a compound or a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 5-170 or a derivative, or a fragment thereof, or complementary thereto (separate or enclosed in a vector) for the manufacture of a medicament for the treatment of a pathological condition involving cognitive impairment or a susceptibility to the condition.

Another aspect of the invention relates to a method for treatment, prevention or amelioration of a pathological condition involving cognitive impairment or a susceptibility to the condition, which comprises administration to a subject a compound or pharmaceutical acceptable salt or derivative thereof, or a vector encoding such compound, which compound inhibits the activity of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and 4 or a derivative or a fragment thereof.

Yet another aspect of the invention relates to a method of reducing or inhibiting the level of amyloid-beta peptide 11-42 or 11-40, 1-42 or 1-40 in a subject, comprising contacting said subject with a compound according to the invention or a pharmaceutical acceptable salt or derivative thereof, which compound inhibits the

activity of polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and 4 or a functional derivative or fragment thereof.

A further aspect of the invention relates to a pharmaceutical composition comprising a compound according to the present invention further comprising a pharmaceutical acceptable carrier and/or diluent and/or excipient.

A "pharmaceutically acceptable carrier and/or diluent and/or excipient" refers to any useful solvent, dispersion medium, coating, antibacterial and antifungal agent, isotonic and absorption delaying agent, and the like, compatible with pharmaceutical administration. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, Finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. Supplementary active compounds can also be incorporated into the compositions. A pharmaceutical composition of the compound is formulated to be compatible with its intended route of administration, including intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (i.e., topical), transmucosal, and rectal administration.

Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as

acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, CREMOPHOR EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). Such compositions are preferably stable during manufacture and storage and must generally be preserved against contamination from microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (such as glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures. Proper fluidity can be maintained, for example, by using a coating such as lecithin, by maintaining the required particle size in the case of dispersion and by using surfactants. Various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, and thimerosal, can contain microorganism contamination. Isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride can be included in the composition. Compositions that can delay absorption include agents such as aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by

incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients as required, followed by sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium, and the other required ingredients. Sterile powders for the preparation of sterile injectable solutions, methods of preparation include vacuum drying and freeze-drying that yield a powder containing the active ingredient and any desired ingredient from a sterile solution.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included. Tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, PRIMOGEL, or corn starch; a lubricant such as magnesium stearate or STEROTES; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are generally delivered as an aerosol spray from a nebulizer or a pressurized container that contains a suitable

propellant, e.g., a gas such as carbon dioxide.

Systemic administration can also be transmucosal or transdermal. For transmucosal or transdermal administration, penetrants that can permeate the target barrier(s) are generally selected. Transmucosal penetrants include detergents, bile salts, and fusidic acid derivatives. Nasal sprays or suppositories can be used for transmucosal administration. For transdermal administration, the active compounds are generally formulated into ointments, salves, gels, or creams.

The compounds can also be prepared in the form of suppositories (e.g., with bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable or biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Polyethylene glycols, e.g. PEG, are also good carriers. Such materials can be obtained commercially from ALZA Corporation (Mountain View, Calif.) and NOVA Pharmaceuticals, Inc. (Lake Elsinore, Calif.), or prepared by one of skill in the art. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, such as in US 4,522,811.

Oral formulations or parenteral compositions in unit dosage form can be created to facilitate administration and dosage uniformity. Unit dosage form refers to physically discrete units suited as single dosages for

the subject to be treated, containing a therapeutically effective quantity of active compound in association with the required pharmaceutical carrier. The specification for the unit dosage forms of the invention are dictated by, and directly dependent on, the unique characteristics of the active compound and the particular desired therapeutic effect, and the inherent limitations of compounding the active compound.

The invention also relates to a method for diagnosing a pathological condition in a subject comprising comparing the nucleic acid sequence of the subject's mRNA or genomic DNA with a nucleic acid of SEQ ID NO: 1 or 2; and identifying any difference(s) between the nucleic acid sequence of the subject's mRNA or genomic DNA and the nucleic acid of SEQ ID NO: 1 or 2.

The invention also relates to a method for diagnosing a pathological condition in a subject, comprising determining the amount of polypeptide comprising an amino acid sequence of SEQ ID NO: 3 or 4 in a sample from said subject, and comparing the amount with the amount of the polypeptide in a sample of a healthy subject. In such a method, an increase of the amount of polypeptide compared to the healthy subject is indicative of the presence of a pathological condition.

It is well understood in the art that databases such as GenBank, can be searched to identify genomic sequences that contain regions of identity (exons) to a nucleic acid. Such genomic sequences encode for the nucleic acid.

The term "amyloid beta peptide" refers to amyloid beta peptides with different composition that are processed from the amyloid beta precursor protein (APP). Examples of the species comprise 1-40, 1-42, y-42,

whereby y ranges from 1-17, and 1-x whereby x ranges from 24-42, and 11-40 and 11-42.

The term "compound", besides relating to the entities outlined above, also relates to organic and inorganic compounds, such as synthetic molecules, peptides, lipids, antibodies and natural compounds.

The term "agonist" predominantly refers to a ligand that activates the receptor the ligand binds to. However, other types of agonists may be applicable (for instance in the form of 'triggering' antibodies) as long as they stimulate the activation of the receptor.

The term "functional derivatives of a polypeptide" relate to those peptides, oligopeptides, polypeptides, proteins and enzymes that retain the biological activity (functionality) of the protein, e.g. polypeptides that have amino acid mutations compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may further comprise additional naturally occurring, altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally occurring form of the polypeptide. It may also contain one or more non-amino acid substituents compared to the amino acid sequence of a naturally occurring form of the polypeptide, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence.

The term "functional fragment of a polypeptide" relates to peptides, oligopeptides, polypeptides, proteins and enzymes that exhibit substantially a similar, but not necessarily identical, activity as the complete sequence.

The term "polynucleotide" refers also to nucleic acids with modified backbones such as peptide nucleic

acid, polysiloxane, and 2'-O-(2-methoxy) ethylphosphorothioate.

The term "derivatives of a polynucleotide" relates to DNA- and RNA- molecules, nucleic acids, and oligonucleotides that may have nucleic acid mutations compared to the nucleic acid sequence of a naturally occurring form of the polynucleotide. A derivative may further comprise nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethyl-phosphorothioate, non-naturally occurring nucleic acid residues, or one or more nuclei acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection. The term "fragment of a polynucleotide" relates to oligonucleotides that exhibit substantially a similar, but not necessarily identical, activity as the complete sequence.

Compounds such as described in US patent 6,699,873 and US patent 6,693,165 provide examples of compounds that reduce or inhibit the activity of MC4R. Inhibition or reduction of MC4R and MC3R activity has been associated with possible treatment of a variety of diseases, such as obesity, but compounds that reduce or inhibit the activity of MC4R and MC3R have never been associated with treatment or prevention or amelioration of a pathological condition involving cognitive impairment.

TABLE 1. GPCRs involved in APP processing (SEQ ID NO:1 relates to the nucleic acid sequence of MC4R, SEQ ID NO:2 to the nucleic acid sequence of MC3R, SEQ ID NO:3 to the amino acid sequence of MC4R and SEQ ID NO:4 to the amino acid sequence of MC3R). Sequences for the compounds (or the expression inhibitory agents are given in SEQ ID NO:5-105 for MC4R and in SEQ ID NO:106-170 for MC3R). The preferred hairpin loop sequence is provided by SEQ ID NO:171.

Gene symbol	Gene description	Ref Seq accession (DNA)	SEQ ID NO's DNA	Ref Seq accession (Protein)	SEQ ID NO's Protein	SEQ_ID NO's compounds (inhibitory agents)
MC4R	Melanocortin 4 receptor	NM_005912	1	NP_005903	3	5 to 105
MC3R	Melanocortin 3 receptor	NM_019888	2	NP_063941	4	106 to 170

Sequence ID	Sequence	Gene Symbol
SEQ ID NO: 5	CTCTGATGGAGGGTGCTAC	MC4R
SEQ ID NO: 6	GGAGGGTGCTACGAGCAAC	MC4R
SEQ ID NO: 7	CTCCTGAGGTGTTTGTGAC	MC4R
SEQ ID NO: 8	CCTGAGGTGTTTGTGACTC	MC4R
SEQ ID NO: 9	GTTTGTGACTCTGGGTGTC	MC4R
SEQ ID NO: 10	TGTGACTCTGGGTGTCATC	MC4R
SEQ ID NO: 11	GACTCTGGGTGTCATCAGC	MC4R
SEQ ID NO: 12	TAGTGATTGTGGCAATAGC	MC4R
SEQ ID NO: 13	AGTGATTGTGGCAATAGCC	MC4R
SEQ ID NO: 14	TGTGGCAATAGCCAAGAAC	MC4R
SEQ ID NO: 15	GCCAAGAACAAGAATCTGC	MC4R
SEQ ID NO: 16	ACAAGAATCTGCATTCACC	MC4R
SEQ ID NO: 17	CAAGAATCTGCATTCACCC	MC4R
SEQ ID NO: 18	TCTGCATTCACCCATGTAC	MC4R
SEQ ID NO: 19	TTGGCTGTGGCTGATATGC	MC4R
SEQ ID NO: 20	GGCTGATATGCTGGTGAGC	MC4R
SEQ ID NO: 21	ATATGCTGGTGAGCGTTTC	MC4R
SEQ ID NO: 22	TGAGCGTTTCAAATGGATC	MC4R
SEQ ID NO: 23	GAAACCATTATCATCACCC	MC4R
SEQ ID NO: 24	ACAGTACAGATACGGATGC	MC4R
SEQ ID NO: 25	AGTACAGATACGGATGCAC	MC4R
SEQ ID NO: 26	TACGGATGCACAGAGTTTC	MC4R
SEQ ID NO: 27	CGGATGCACAGAGTTTCAC	MC4R
SEQ ID NO: 28	TGTCATTGACTCGGTGATC	MC4R
SEQ ID NO: 29	TGACTCGGTGATCTGTAGC	MC4R
SEQ ID NO: 30	ACTCGGTGATCTGTAGCTC	MC4R
SEQ ID NO: 31	CTCGGTGATCTGTAGCTCC	MC4R
SEQ ID NO: 32	GTGATCTGTAGCTCCTTGC	MC4R
SEQ ID NO: 33	TCTGTAGCTCCTTGCTTGC	MC4R

Sequence ID	Sequence	Gene Symbol
SEQ ID NO: 34	GTAGCTCCTTGCTTGCATC	MC4R
SEQ ID NO: 35	TAGCTCCTTGCTTGCATCC	MC4R
SEQ ID NO: 36	CTTGCTTGCATCCATTTGC	MC4R
SEQ ID NO: 37	GCTTGCATCCATTTGCAGC	MC4R
SEQ ID NO: 38	CTTGCATCCATTTGCAGCC	MC4R
SEQ ID NO: 39	GCATCCATTTGCAGCCTGC	MC4R
SEQ ID NO: 40	CCATTTGCAGCCTGCTTTC	MC4R
SEQ ID NO: 41	GCAGCCTGCTTTCAATTGC	MC4R
SEQ ID NO: 42	GCTTTCAATTGCAGTGGAC	MC4R
SEQ ID NO: 43	AATTGCAGTGGACAGGTAC	MC4R
SEQ ID NO: 44	CAGTGGACAGGTACTTTAC	MC4R
SEQ ID NO: 45	GGACAGGTACTTTACTATC	MC4R
SEQ ID NO: 46	CTTCTATGCTCTCCAGTAC	MC4R
SEQ ID NO: 47	TTCTATGCTCTCCAGTACC	MC4R
SEQ ID NO: 48	TGCTCTCCAGTACCATAAC	MC4R
SEQ ID NO: 49	AGTTAAGCGGGTTGGGATC	MC4R
SEQ ID NO: 50	TAAGCGGGTTGGGATCATC	MC4R
SEQ ID NO: 51	TCATAAGTTGTATCTGGGC	MC4R
SEQ ID NO: 52	TAAGTTGTATCTGGGCAGC	MC4R
SEQ ID NO: 53	TTGTATCTGGGCAGCTTGC	MC4R
SEQ ID NO: 54	GTATCTGGGCAGCTTGCAC	MC4R
SEQ ID NO: 55	CTCAGATAGTAGTGCTGTC	MC4R
SEQ ID NO: 56	AGATAGTAGTGCTGTCATC	MC4R
SEQ ID NO: 57	TAGTAGTGCTGTCATCATC	MC4R
SEQ ID NO: 58	TAGTGCTGTCATCATCTGC	MC4R
SEQ ID NO: 59	AGTGCTGTCATCATCTGCC	MC4R
SEQ ID NO: 60	TGCTGTCATCATCTGCCTC	MC4R
SEQ ID NO: 61	TGTCATCATCTGCCTCATC	MC4R
SEQ ID NO: 62	TCATCATCTGCCTCATCAC	MC4R
SEQ ID NO: 63	CATCATCTGCCTCATCACC	MC4R
SEQ ID NO: 64	CTGCCTCATCACCATGTTC	MC4R
SEQ ID NO: 65	CCTCATCACCATGTTCTTC	MC4R
SEQ ID NO: 66	TCATCACCATGTTCTTCAC	MC4R
SEQ ID NO: 67	CATCACCATGTTCTTCACC	MC4R
SEQ ID NO: 68	ACCATGTTCTTCACCATGC	MC4R
SEQ ID NO: 69	TGTTCTTCACCATGCTGGC	MC4R
SEQ ID NO: 70	TTCTTCACCATGCTGGCTC	MC4R
SEQ ID NO: 71	CTTCACCATGCTGGCTCTC	MC4R
SEQ ID NO: 72	TGCTGGCTCTCATGGCTTC	MC4R
SEQ ID NO: 73	CTGGCTCTCATGGCTTCTC	MC4R
SEQ ID NO: 74	GGCTCTCATGGCTTCTCTC	MC4R
SEQ ID NO: 75	CATGGCTTCTCTCTATGTC	MC4R
SEQ ID NO: 76	ATGGCTTCTCTCTATGTCC	MC4R
SEQ ID NO: 77	GGCTTCTCTCTATGTCCAC	MC4R
SEQ ID NO: 78	TCTCTATGTCCACATGTTC	MC4R
SEQ ID NO: 79	CTCTATGTCCACATGTTCC	MC4R
SEQ ID NO: 80	TCCACATGTTCCTGATGGC	MC4R
SEQ ID NO: 81	CCACATGTTCCTGATGGCC	MC4R
SEQ ID NO: 82	ATGTTCCTGATGGCCAGGC	MC4R
SEQ ID NO: 83	TTCCTGATGGCCAGGCTTC	MC4R

Sequence ID	Sequence	Gene Symbol
SEQ ID NO: 84	CCTGATGGCCAGGCTTCAC	MC4R
SEQ ID NO: 85	TTCACATTAAGAGGATTGC	MC4R
SEQ ID NO: 86	CATTAAGAGGATTGCTGTC	MC4R
SEQ ID NO: 87	ATTAAGAGGATTGCTGTCC	MC4R
SEQ ID NO: 88	TAAGAGGATTGCTGTCCTC	MC4R
SEQ ID NO: 89	AAGAGGATTGCTGTCCTCC	MC4R
SEQ ID NO: 90	AGAGGATTGCTGTCCTCCC	MC4R
SEQ ID NO: 91	GTGCCAATATGAAGGGAGC	MC4R
SEQ ID NO: 92	ATATGAAGGGAGCGATTAC	MC4R
SEQ ID NO: 93	TATGAAGGGAGCGATTACC	MC4R
SEQ ID NO: 94	AGGGAGCGATTACCTTGAC	MC4R
SEQ ID NO: 95	GGGAGCGATTACCTTGACC	MC4R
SEQ ID NO: 96	AGCGATTACCTTGACCATC	MC4R
SEQ ID NO: 97	GCGATTACCTTGACCATCC	MC4R
SEQ ID NO: 98	CTTGACCATCCTGATTGGC	MC4R
SEQ ID NO: 99	GACCATCCTGATTGGCGTC	MC4R
SEQ ID NO: 100	GATTGGCGTCTTTGTTGTC	MC4R
SEQ ID NO: 101	TGGCGTCTTTGTTGTCTGC	MC4R
SEQ ID NO: 102	TCTTTGTTGTCTGCTGGGC	MC4R
SEQ ID NO: 103	CTTTGTTGTCTGCTGGGCC	MC4R
SEQ ID NO: 104	TTTGTTGTCTGCTGGGCCC	MC4R
SEQ ID NO: 105	ATCTCTTGTCCTCAGAATC	MC4R
SEQ ID NO: 106	TTCTGACAGCAATGAATGC	MC3R
SEQ ID NO: 107	TGACAGCAATGAATGCTTC	MC3R
SEQ ID NO: 108	AGCAATGAATGCTTCGTGC	MC3R
SEQ ID NO: 109	AATGAATGCTTCGTGCTGC	MC3R
SEQ ID NO: 110	ATGAATGCTTCGTGCTGCC	MC3R
SEQ ID NO: 111	AATGCTTCGTGCTGCCTGC	MC3R
SEQ ID NO: 112	CCCTCTGTTCAGCCAACAC	MC3R
SEQ ID NO: 113	TCTGTTCAGCCAACACTGC	MC3R
SEQ ID NO: 114	CTGTTCAGCCAACACTGCC	MC3R
SEQ ID NO: 115	GCCAACACTGCCTAATGGC	MC3R
SEQ ID NO: 116	CAACACTGCCTAATGGCTC	MC3R
SEQ ID NO: 117	TTTCTTCAGCAACCAGAGC	MC3R
SEQ ID NO: 118	CTTCAGCAACCAGAGCAGC	MC3R
SEQ ID NO: 119	CAGCAACCAGAGCAGCAGC	MC3R
SEQ ID NO: 120	CTTCTGTGAGCAGGTCTTC	MC3R
SEQ ID NO: 121	CTGTGAGCAGGTCTTCATC	MC3R
SEQ ID NO: 122	GAGCAGGTCTTCATCAAGC	MC3R
SEQ ID NO: 123	AGCAGGTCTTCATCAAGCC	MC3R
SEQ ID NO: 124	GCAGGTCTTCATCAAGCCC	MC3R
SEQ ID NO: 125	CCTGTCTCTGGGCATCGTC	MC3R
SEQ ID NO: 126	TCTCTGGGCATCGTCAGTC	MC3R
SEQ ID NO: 127	ACATCCTGGTTATCCTGGC	MC3R
SEQ ID NO: 128	CATCCTGGTTATCCTGGCC	MC3R
SEQ ID NO: 129	GATGTACTTCTTTCTCTGC	MC3R
SEQ ID NO: 130	GTACTTCTTTCTCTGCAGC	MC3R
SEQ ID NO: 131	TACTTCTTTCTCTGCAGCC	MC3R
SEQ ID NO: 132	TCTTTCTCTGCAGCCTGGC	MC3R
SEQ ID NO: 133	ACATGCTGGTAAGTGTGTC	MC3R

Sequence ID	Sequence	Gene Symbol
SEQ ID NO: 134	CATGCTGGTAAGTGTGTCC	MC3R
SEQ ID NO: 135	TGGTAAGTGTGTCCAATGC	MC3R
SEQ ID NO: 136	GGTAAGTGTGTCCAATGCC	MC3R
SEQ ID NO: 137	GTAAGTGTGTCCAATGCCC	MC3R
SEQ ID NO: 138	TGTCCAATGCCCTGGAGAC	MC3R
SEQ ID NO: 139	CAATGCCCTGGAGACCATC	MC3R
SEQ ID NO: 140	CCTGGAGACCATCATGATC	MC3R
SEQ ID NO: 141	TGGAGACCATCATGATCGC	MC3R
SEQ ID NO: 142	GGAGACCATCATGATCGCC	MC3R
SEQ ID NO: 143	GACCATCATGATCGCCATC	MC3R
SEQ ID NO: 144	CATCATGATCGCCATCGTC	MC3R
SEQ ID NO: 145	ATCATGATCGCCATCGTCC	MC3R
SEQ ID NO: 146	CATGATCGCCATCGTCCAC	MC3R
SEQ ID NO: 147	GATCGCCATCGTCCACAGC	MC3R
SEQ ID NO: 148	CAGCGACTACCTGACCTTC	MC3R
SEQ ID NO: 149	CTACCTGACCTTCGAGGAC	MC3R
SEQ ID NO: 150	TACCTGACCTTCGAGGACC	MC3R
SEQ ID NO: 151	CTTCGAGGACCAGTTTATC	MC3R
SEQ ID NO: 152	TTCGAGGACCAGTTTATCC	MC3R
SEQ ID NO: 153	GAGGACCAGTTTATCCAGC	MC3R
SEQ ID NO: 154	GGACCAGTTTATCCAGCAC	MC3R
SEQ ID NO: 155	GTTTATCCAGCACATGGAC	MC3R
SEQ ID NO: 156	CATCTTCGACTCCATGATC	MC3R
SEQ ID NO: 157	TCCATGATCTGCATCTCCC	MC3R
SEQ ID NO: 158	CCTCTACGTGCACATGTTC	MC3R
SEQ ID NO: 159	ATGTTCCTCTTTGCGCGGC	MC3R
SEQ ID NO: 160	GTCAAGCGCATAGCAGCAC	MC3R
SEQ ID NO: 161	CATCACCATTCTCCTGGGC	MC3R
SEQ ID NO: 162	CATTCTCCTGGGCGTGTTC	MC3R
SEQ ID NO: 163	TGCATCTGCTACACTGCCC	MC3R
SEQ ID NO: 164	TGCCCACTTCAACACCTAC	MC3R
SEQ ID NO: 165	TTCAACACCTACCTGGTCC	MC3R
SEQ ID NO: 166	CACCTACCTGGTCCTCATC	MC3R
SEQ ID NO: 167	CTCCGTCATCGACCCACTC	MC3R
SEQ ID NO: 168	CCACTCATCTACGCTTTCC	MC3R
SEQ ID NO: 169	CACCTTTAGGGAGATTCTC	MC3R
SEQ ID NO: 170	CCTCATCACCATGTTCTTC	MC3R
SEQ ID NO: 171	GTTTGCTATAAC	Loop sequence

TABLE2: buffers and solutions used for ELISA

Buffer 42	30mM $NaHCO_3$, 70mM Na_2CO_3, 0.05% NaN_3, pH9.6
Casein buffer	0.1% casein in PBS 1x
EC Buffer	20mM sodium phosphate, 2mM EDTA, 400mM NaCl, 0.2% BSA, 0.05% CHAPS, 0.4% casein, 0.05% NaN_3, pH7
Buffer C	20mM sodium phosphate, 2mM EDTA, 400mM NaCl, 1% BSA, pH7
PBS 10x	80g NaCl + 2g KCl + 11.5g $Na_2HPO_4.7H_2O$ + 2g KH_2PO_4 in 1 l milli Q, pH 7.4
PBST	PBS 1x with 0.05% Tween 20

TABLE 3: Primers used in the quantitative real time PCR analysis for MC4R expression levels.

gene	Primer name	SEQ ID NO:	Primer sequence
MC4R	MC4R_Hs_For	172	GGAACCGCAGCAGTTACAGACT
	MC4R_Hs_Rev	173	TCGTAGCACCCTCCATCAGAGT

TABLE 4: Ct values obtained during quantitative real time PCR: human cerebral cortex or human hippocampus RNA is tested for the presence of MC4R mRNA via quantitative real time PCR. GAPDH RNA is used to normalize all samples (ΔCt).

Human Tissue	GAPDH Ct-values		MC4R Ct-values		ΔCt (+RT)
	+RT	-RT	+RT	-RT	
Hippocampus	16.11	33.07	27.01	36.50	10.90
Cerebral cortex	15.68	39.63	26.66	40	10.98

EXAMPLES

EXAMPLE 1. MC4R increases amyloid beta 1-42 levels.

To identify novel drug targets that change the APP processing, a stable cell line overexpressing APP, Hek293 APPwt, was transduced with adenoviral cDNA libraries and the resulting amyloid beta 1-42 levels were detected via ELISA. This stable cell line was created after transfection of Hek293 cells with the APP770wt cDNA cloned in pcDNA3.1 and selection with G418 during 3 weeks. At this time point colonies were picked and stable clones were expanded and tested for their secreted amyloid beta peptide levels.

The assay was performed as follows. Cells seeded in collagen-coated plates at a cell density of 15,000 cells/well (384 well plate) in DMEM 10%FBS, were infected 24 h later with 1 µl or 0.2 µl of adenovirus (corresponding to an average multiplicity of infection (MOI) of 120 and 24 respectively). The following day, the virus was washed away and DMEM 25 mM Hepes 10%FBS was added to the cells. Amyloid beta peptides were allowed to accumulate during 24h. The ELISA plate was prepared by coating the capture antibody (JRF/cAbeta42/26) (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium) overnight in buffer 42 (table 2) at a concentration of 2,5 µg/ml. The excess capture antibody was washed away the next morning with PBS and the ELISA plate was then blocked overnight with casein buffer (table 2) at 4°C. Upon removal of the blocking buffer, 30 µl of the sample was transferred to the ELISA plate and incubated overnight at 4°C. After extensive washing with PBS-Tween20 and PBS, 30 µl of the horse reddish peroxidase (HRP) labeled detection antibody (Peroxidase Labeling

Kit, Roche), JRF/AbetaN/25-HRP (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium) was diluted 1/5000 in buffer C (table 2) and added to the wells for another 2h. Following the removal of excess detection antibody by a wash with PBS-Tween20 and PBS, HRP activity was detected via addition of luminol substrate (Roche), which was converted into a chemiluminescent signal by the HRP enzyme.

In order to validate the assay, the effect of adenoviral overexpression with random titre of two clinical PS1 mutants and BACE on amyloid beta 1-42 production was evaluated in the Hek293 APPwt cells. As is shown in Figure 2, all constructs induce amyloid beta 1-42 levels as expected.

An adenoviral cDNA library containing almost all GPCRs was constructed as follows. DNA fragments covering the full coding region of the GPCRs, were amplified by PCR from a pooled placental and fetal liver cDNA library (InvitroGen). All fragments were cloned into an adenoviral vector as disclosed in US 6,340,595 and subsequently adenoviruses were made harboring the corresponding cDNAs. This GPCR library was screened according the above-described procedure with the exception that during the 24 h. A specific agonist for each receptor was added. Every non-orphan GPCR was screened in quadruple with four different agonist concentrations (250 nM, 25 nM, 2.5 nM and in the absence). During the screening of the adenoviral GPCR library in the Hek293 APPwt cells, MC4R was identified as a modulator of APP processing. These results indicate that overexpression and activation of MC4R leads to increased levels of amyloid beta 1-42 peptides in the

conditioned medium of Hek293 APPwt cells, showing that this GPCR modulates APP processing.

The stimulatory effect of MC4R was confirmed upon re-screening of the viruses with a known titer (viral particles/ml), as determined by quantitative real time PCR. MC4R virus was infected at MOIs ranging from 2 to 250 and the experiment was performed as described above. Amyloid beta 1-42 levels were 2 fold higher compared to the negative controls for Ad5/MC4R (Figure 3A). In addition, the effect of MC4R on amyloid beta 1-40, 11-42, 1-x and y-42 levels were checked under similar conditions as above (Figure 3B). The respective ELISA's were performed as described above, except that the following antibodies were used: for the amyloid beta 1-40 ELISA, the capture and detection antibody were respectively JRF/cAbeta40/10 and JRF/AbetaN/25-HRP (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium), for the amyloid beta 11-42 ELISA, the capture and detection antibody were respectively JRF/cAbeta42/26 and JRF/hAb11/1 (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium), for the amyloid beta y-42 ELISA (y ranges from 1-17), the capture and detection antibody were respectively JRF/cAbeta42/26 and 4G8-HRP (obtained respectively from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium and from Signet, USA) while for the amyloid beta 1-x ELISA (x ranges from 24-42) the capture and detection antibodies were JRF/AbetaN/25 and 4G8-HRP, respectively (obtained respectively from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium and from Signet, USA). The amyloid beta 1-x ELISA was used for the detection of amyloid peptides with a

variable C-terminus (amyloid beta 1-37; 1-38; 1-39; 1-40; 1-42). The results of these experiments clearly show an increase of amyloid beta 1-40, 11-42, y-42 and 1-x species upon transduction of MC4R (figure 3B-3E). The same procedure is used for the analysis of APP processing by MC3R.

Example 2. Modulation of amyloid beta 1-42 production in HEK293APPwt cells infected with MC4R adenovirus by MC4R ligands.

Demonstrating that the secretion of beta amyloid was modulated by specific ligands of MC4R shows that this GPCR is specifically involved in the secretion of beta amyloid and that interference with the signal transduction pathway of this GPCR affects the beta amyloid secretion. Overexpression of MC4R in HEK293APPwt cells increased the amount of beta amyloid 1-42 secreted in the medium as determined by the ELISA identical to the ELISA used for screening. Stimulation of the MC4R receptor with the MC4R agonist alpha-MSH dose dependently increased the beta amyloid secretion further (Figure 4A). These increased levels of beta amyloid 1-42 are dose dependently decreased by treating the cells with the antagonist SHU9119 (Figure 4A). The increased basal levels of beta amyloid secretion due to MC4R overexpression were dose dependently reduced by treatment of the cells with Agouti related peptide (Figure 4B) (inverse agonist for MC4R).

EXAMPLE 3. Expression of MC4R in the human brain.

Upon identification of a modulator of APP processing, it is important to evaluate whether the modulator is expressed in the tissue and the cells of interest. This can be achieved by measuring the RNA

and/or protein levels. In recent years, RNA levels were being quantified through real time PCR technologies, whereby the RNA is first transcribed to cDNA and then the amplification of the cDNA of interest is monitored during a PCR reaction. The amplification plot and the resulting Ct value are indicators for the amount of RNA present in the sample. Determination of the levels of household keeping genes allows the normalization of RNA levels of the target gene between different RNA samples, represented as delta Ct values.

To assess whether the GPCR of the invention is expressed in the human brain, real time PCR with GAPDH specific primers and specific primers for GPCR of the invention was performed on human cerebral cortex and human hippocampal total RNA (BD Biosciences). GAPDH was detected with a Taqman probe, while for the GPCR SybrGreen was used. In short, 40 ng of RNA was transcribed to DNA using the MultiScribe Reverse Transcriptase (50 U/µl) enzyme (Applied BioSystems). The resulting cDNA was amplified with AmpliTaq Gold DNA polymerase (Applied BioSystems) during 40 cycles using an ABI PRISM® 7000 Sequence Detection System. Cerebral cortex and hippocampal total RNA's were analyzed for the presence of the GPCR transcripts via quantitative real time PCR. For MC4R, the obtained Ct values indicate that it was detected in all RNA samples (table 4).

To gain more insight into the specific cellular expression, immunohistochemistry (protein level) and/or *in situ* hybridization (RNA level) were carried out on sections from human normal and Alzheimer's brain hippocampal, cortical and subcortical structures. These results indicate whether expression occurs in neurons, microglia cells or astrocytes. The comparison of diseased tissue with healthy tissue, teaches us whether MC4R is

expressed in the diseased tissue and whether its expression level is changed compared to the non-pathological situation. The same procedure is used for expression profiling of MC3R.

EXAMPLE 4. Amyloid beta production in rat primary neuronal cells.

In order to investigate whether MC4R of the invention affects amyloid beta production in a real neuron, human or rat primary hippocampal or cortical neurons are treated with ligands for the MC4R. Amyloid beta levels are determined by ELISA (see EXAMPLE 1). A rodent specific beta amyloid antibody is used for the detection.

Human primary neurons are purchased from Cellial Technologies, France. Rat primary neuron cultures are prepared from brain of E18-E19-day-old fetal Sprague Dawley rats according to Goslin and Banker (Culturing Nerve cells, second edition, 1998 ISBN 0-262-02438-1). Briefly, single cell suspensions obtained from the hippocampus or cortices are prepared. The number of cells is determined (only taking into account the living cells) and cells are plated on poly-L-lysine-coated plastic 96-well plates in minimal essential medium (MEM) supplemented with 10% horse serum. The cells are seeded at a density between 30,000 and 60,000 cells per well (i.e. about 100,000 - 200,000 cells/cm^2, respectively). After 3-4 h, culture medium is replaced by 150 µl serum-free neurobasal medium with B27 supplement (GIBCO BRL). Cytosine arabinoside (5 µM) is added 24 h after plating to prevent nonneuronal (glial) cell proliferation.

Neurons are used at day 5-7 after plating, 150 µl conditioned medium of these cultures is transferred to the corresponding wells in an empty 96-well plate and 50

µl of the conditioned medium returns to the cells. The remaining 100 µl/well is stored at 37°C and 5% CO_2. Cultures are washed with 100 µl pre-warmed fresh neurobasal medium. After removal of the wash solution, the remaining 100 µl of the stored conditioned medium is transferred to the corresponding cells. Ligands are added to the medium in maximal effective doses. From now on, cells accumulate amyloid beta in the conditioned medium and its concentration is determined by amyloid beta 1-42 and amyloid beta x-42 specific ELISA's (see EXAMPLE 1). The conditioned media are collected 24, 48 and 96 hours after addition of the stored conditioned medium.

In addition, the procedure as described above is used to analyze rodent amyloid beta by means of an ELISA protocol described in EXAMPLE 1. The ELISA plate is prepared by coating the capture antibody (JRF/cAbeta42/26) (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium) and amyloid beta levels is determined by the horse reddish peroxidase (HRP) labeled detection antibody (Peroxidase Labeling Kit, Roche), JRF/rAb/2-HRP (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium). The same procedure is used for the analysis of MC3R. Co-infection with human APP is considered.

EXAMPLE 5. Ligand screens for GPCRs.

Reporter gene screen.

Mammalian cells such as HEK293 or CHO-K1 cells are either stably transfected with a plasmid harboring the luciferase gene under the control of a cAMP dependent promoter (CRE elements) or transduced with an adenovirus harboring a luciferase gene under the control of a cAMP dependent promoter. In addition other reporter constructs

can be used with the luciferase gene under the control of a Ca^{2+} dependent promoter (NF-AT elements) or a promoter that is controlled by activated NF-κB. These cells, expressing the reporter construct, are then transduced with an adenovirus harboring the cDNA of the GPCR of the present invention. 24 to 40 h after transduction the cells are treated with.

An agonist for the receptor (e.g. alpha-MSH or MTII) and screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris, Biofocus, Evotec), or

Compounds, which decrease the agonist induced increase in luciferase activity, are considered to be antagonists or inverse agonists for the MC4R. These compounds are screened again for verification and screened against their effect on secreted amyloid beta peptide levels.

In addition, cells expressing the NF-AT reporter gene can be transduced with an adenovirus harboring the cDNA encoding the α-subunit of G-alpha15 or chimerical Gα subunits. G_{15} is a promiscuous G protein of the G_q class that couples to many different GPCRs and as such re-directs their signaling towards the release of intracellular Ca^{2+} stores. The chimerical G alpha subunits are members of the G_s and $G_{i/o}$ family by which the last 5 C-terminal residues are replaced by those of Gαq, these chimerical G-proteins also redirect cAMP signaling to Ca^{2+} signaling.

FLIPR screen.

Mammalian cells such as HEK293 or CHO-K1 cells are stably transfected with an expression plasmid construct

harboring the cDNA of a GPCR of the present invention. Cells are seeded and grown until sufficient stable cells can be obtained. Cells are loaded with a Ca^{2+} dependent fluorophore such as Fura3 or Fura4. After washing away the excess of fluorophore the cells are screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris, Biofocus, Evotec) by simultaneously adding an agonist and a compound to the cells. As a reference just the agonist is added. Activation of the receptor is measured as an almost instantaneously increase in fluorescence due to the interaction of the fluorophore and the Ca^{2+} that is released. Compounds that reduce or inhibit the agonist induced increase in fluorescence are considered to be antagonists or inverse agonists for the receptor they are screened against. These compounds will be screened again to measure the effect on secreted amyloid beta peptide.

AequoScreen.

CHO cells, stably expressing Apoaequorin are stably transfected with a plasmid construct harboring the cDNA of a GPCR. Cells are seeded and grown until sufficient stable cells can be obtained. The cells are loaded with coelenterazine, a cofactor for apoaequorin. Upon receptor activation intracellular Ca^{2+} stores will be emptied and the aequorin will react with the coelenterazine in a light emitting process. The emitted light is a measure for receptor activation. The CHO, stable expressing both the apoaequorin and the receptor are screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural

compounds (Specs, TimTech), and small chemical compounds (Tocris, Biofocus, Evotec, Euroscreen) by simultaneously adding an agonist and a compound to the cells or by only adding a compound. As a reference just the agonist is added. Activation of the receptor is measured as an almost instantaneously light flash due to the interaction of the apoaequorin, coelenterazine and the Ca^{2+} that is released. Compounds that reduce or inhibit the agonist induced increase in light are considered to be antagonists or inverse agonists for the receptor they are screened against. These compounds will be screened again for verification and effect they have on secreted amyloid beta levels.

In addition, CHO cells stable expressing the apoaequorin gene are stably transfected with a plasmid construct harboring the cDNA encoding the α-subunit of G_{15} or chimerical G_α subunits. G_{15} is a promiscuous G protein of the G_q class that couples to many different GPCRs and as such redirect their signaling towards the release of intracellular Ca^{2+} stores. The chimerical G alpha subunits are members of the G_s and $G_{i/o}$ family by which the last 5 C-terminal residues are replaced by those of $G_{\alpha q}$, these chimerical G-proteins also redirect cAMP signaling to Ca^{2+} signaling.

Screening for compounds that interact with MC3R and/or MC4R (displacement experiment)

Compounds are screened for binding. The affinity of the compounds to the polypeptides is determined in a displacement experiment. In brief, the polypeptides of the present invention are incubated with a labeled (radiolabeled, fluorescent labeled) ligand that is known to bind to the polypeptide and with an unlabeled compound. The displacement of the labeled ligand from the

polypeptide is determined by measuring the amount of labeled ligand that is still associated with the polypeptide. The amount associated with the polypeptide is plotted against the concentration of the compound to calculate IC_{50} values. This value reflects the binding affinity of the compound to its target, i.e. the polypeptides of the present invention. Strong binders have an IC_{50} in the nanomolar and even picomolar range. Compounds that have an IC_{50} of at least 10 micromol or less (nmol to pmol) are applied in beta amyloid secretion assay to check for their effect on the beta amyloid secretion and processing. The polypeptides of the present invention can be prepared in a number of ways depending on whether the assay will be run on cells, cell fractions or biochemically, on purified proteins.

Screening for compounds that interact with MC3R and/or MC4R (generic GPCR screening assay)

When a G protein receptor is activated, it binds to a G protein (Gq, Gs, Gi, Go) and stimulates the binding of GTP to the G protein. The G protein then acts as a GTPase and slowly hydrolyses the GTP to GDP, whereby the receptor, under normal conditions, becomes deactivated. A non-hydrolyzable analog of GTP, $[^{35}S]GTP\gamma S$, can be used to monitor GTP binding to membrane fractions isolated from cells that express a specific GPCR. It is reported that $[^{35}S]GTP\gamma S$ can be used to monitor G protein coupling to membranes in the absence and presence of ligand. Moreover, a preferred approach is the use of Antibodies specific for the various members of the G-alpha-protein family to immune precipitate the G-alpha-protein together with the radiolabelled $[^{35}S]GTP\gamma S$. The amount of $[^{35}S]GTP\gamma S$ incorporated in the imuunprecipitate is then a direct measure of the degree of receptor activation. Homogenized

membranes with MC4R protein are transferred in a 96-well plate. A pin-tool is used to transfer a candidate compound in each well plus [^{35}S]GTPγS, followed by an incubation on a shaker for 60 minutes at room temperature. The assay is stopped by spinning of the plates at 4000 RPM for 15 minutes at 22°C. The membranes are re-suspended and solubilized and the G-alpha-proteins are immunprecipitated. The beads are collected and the amount of radioactivity is determined by scintillation counting. Alternatively scintillation proximity beads from various suppliers can be used for the determination of the amount of radioactivity incorporated. The same procedure is used for analysis of MC3R.

Receptor-ligand binding study on cell surface

The receptor is expressed in mamalian cells (HEK293, CHO, COS7) by adenovirally transducing the cells (see US 6,340,595). The cells are incubated with both labeled ligand (iodinated, tritiated, or fluorescent) and the unlabeled compound at various concentrations, ranging from 10 pM to 10μM (3 hours at 4°C.: 25 mM HEPES, 140 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$ and 0.2% BSA, adjusted to pH 7.4). Reactions mixtures are rapidly aspirated onto PEI-treated GF/B glass filters using a cell harvester (Packard). The filters are washed twice with ice-cold wash buffer (25 mM HEPES, 500 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, adjusted to pH 7.4). Scintillant (MicroScint-10; 35 ìl) is added to dried filters and the filters counted in a (Packard Topcount) scintillation counter. Data are analyzed and plotted using Prism software (GraphPad Software, San Diego, Calif.). Competition curves are analyzed and IC_{50} values calculated. If one or more datapoints do not fall within the sigmoidal range of the competition curve or close to the sigmoidal range the

assay is repeated and concentrations of labeled ligand and unlabeled compound adapted to have more data points close to or in the sigmoidal range of the curve.

Receptor-ligand binding studies on membrane preparations

Membrane preparations are isolated from mammalian cells (HEK293, CHO, COS7) overexpressing the receptor and this is done as follows: Medium is aspirated from the transduced cells and cells are harvested in 1 x PBS by gentle scraping. Cells are pelleted (2500 rpm 5 min) and resuspended in 50 mM Tris pH 7.4 (10 x 10E6 cells/ml). The cell pellet is homogenized by sonicating 3 x 5 sec (UP50H; sonotrode MS1; max amplitude: 140 µm; max Sonic Power Density: 125W/cm^2). Membrane fractions are prepared by centrifuging 20 min at maximal speed (13000 rpm ~15 000 to 20 000g or rcf). The resulting pellet is resuspended in 500 µl 50 mM Tris pH 7.4 and sonicated again for 3 x 5 sec. The membrane fraction is isolated by centrifugation and finally resuspended in PBS. Binding competition and derivation of IC_{50} values are determined as described above.

Internalisation screen (1)

Activation of a GPCR-associated signal transduction pathway commonly leads to translocation of specific signal transduction molecules from the cytoplasma to the plasma membrane or from the cytoplasma to the nucleus. Norak has developed their transfluor assay based on agonist-induced translocation of receptor-â-arrestin-GFP complex from the cytosol to the plasma membrane and subsequent internalization of this complex, which occurs during receptor desensitization. A similar assay uses GFP tagged receptor instead of â-arrestin. HEK293 cells are transduced with a MC4R-eGFP vector that translates for a

MC4R-eGFP fusion protein. 48 hours after transduction, the cells are set to fresh serum-free medium for 60 minutes and treated with a ligand (e.g. 100 nM alpha-MSH or MTII) for 15, 30, 60 or 120 minutes at 37°C and 5% CO_2. After indicated exposure times, cells are washed with PBS and fixed with 5% paraformaldehyde for 20 minutes at RT. GFP fluorescence is visualized with a Zeiss microscope with a digital camera. This method aims for the identification of compounds that inhibit a ligand-mediated (constitutive activity-mediated) translocation of the fusion protein to intracellular compartments. The same procedure is used for analysis of MC3R.

Internalisation screen (2)

Various variations on translocation assays exists using â-arrestin and â-galactosidase enzyme complementation and BRET based assays with receptor as energy donor and â-arrestin as energy acceptor. Also the use of specific receptor antibodies labeled with pH sensitive dyes are used to detect agonist induced receptor translocation to acidic lysosomes. All of he translocation assays are used for screening for both agonistic and antagonistic acting ligands.

Melanophore assay (Arena Pharmaceutical)

The melanophore assay is based on the ability of GPCRs to alter the distribution of melanin cotaining melanosomes in Xenopus melanophores. The distribution of the melanosomes depends on the exogenous receptor that is either Gi/o or Gs/q coupled. The distribution of the melanosomes (dispersed or aggregated) is easily detected by measuring light absorption. This type of assay is used for both agonist as well as antagonist compound screens.

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CLAIMS

1. Method for identifying a compound that influences the processing of an amyloid-beta precursor protein, said method comprising the steps of:

(a) expressing a polypeptide of SEQ ID NO: 3 or 4, or a functional fragment or derivative thereof, in a cell;

(b) determining a first level of processing of an amyloid-beta precursor protein in said cell;

(c) exposing said cell to a compound;

(d) determining a second level of processing of said amyloid-beta precursor protein in said cell; and

(e) identifying the compound, that influences the processing of said amyloid-beta precursor protein.

2. Method for identifying a compound that influences the processing of an amyloid-beta precursor protein, said method comprising the steps of:

(a) expressing a polypeptide selected from the group consisting of SEQ ID NO: 3 or 4, or a functional fragment or derivative thereof, in a cell;

(b) determining a first activity level of said precursor protein;

(c) exposing said cell to a compound;

(d) determining a second activity level of said precursor protein; and

(e) identifying the compound that influences the activity level.

3. Method for identifying a compound that decreases the expression or activity of a polypeptide selected from SEQ ID NO: 3 or 4 in a cell, said method comprising the steps of:

(a) incubating a cell;

(b) determining a first level of expression or activity of said polypeptide in said cell;

(c) incubating said cell with a compound;

(d) determining a second level of expression or activity in said cell of said polypeptide following or during step (c); and

(e) identifying a compound that decreases the expression or activity of said polypeptide.

4. Method according to any one of claims 1-3, wherein the polypeptide is MC4R (SEQ ID NO:3).

5. Method according to any one of claims 1-4, wherein the influence on the processing of said precursor protein or the decrease in expression of the polypeptide is such that the level of amyloid-beta peptide 1-42, 1-40, 11-42 or 11-40 is reduced.

6. Method according to claim 2, wherein said second activity level is lower than the first activity level, preferably wherein the second activity level is close to zero.

7. Method according to any one of claims 1-6, further comprising the step of exposing said cell to an agonist of the polypeptide.

8. Method according to claim 7, wherein the agonist is selected from the group consisting of: alpha-MSH, beta-MSH, gamma-MSH, beta-LPH, gamma-LPH, ACTH, Ro 27-3225 and MTII.

9. Method according to claim 2, wherein the activity is measured by determining the level of the second messengers cyclic AMP or Ca^{2+}.

10. Method according to claim 9, wherein the activity level of at least one second messenger is determined with a reporter gene under the control of a promoter that is responsive to the second messenger.

11. Method according to claim 10, wherein the promoter is a cyclic AMP-responsive promoter, an NF-KB responsive promoter, or a NF-AT responsive promoter.

12. Method according to claim 10 or 11, wherein the reporter gene is selected from the group consisting of: alkaline phosphatase, GFP, eGFP, dGFP, luciferase and β-galactosidase.

13. Method according to any one of claims 1-12, wherein the compound exhibits a binding affinity to the polypeptide of at most 10 micromolar.

14. Method according to any of the claims 1-13, wherein the compound is selected from the group consisting of: a small molecule (low molecular weight) compound, an antisense RNA, an antisense oligodeoxynucleotide (ODN), an siRNA, a ribozyme, an RNAi, an antibody, a nanobody, a peptide, a polypeptide, a nucleic acid, a lipid, a natural compound.

15. Method according to any one of claims 1-14, wherein said compound is an expression inhibitory agent that inhibits the expression and/or the translation of the nucleic acid encoding the polypeptide.

16. Method according to any one of claims 1-15, wherein the compound is provided by a vector.

17. Method according to claim 16, wherein the vector is an adenovirus, a retrovirus, an alphavirus, an adeno-associated virus (AAV), a lentivirus, a herpes simplex virus (HSV) or a sendai virus.

18. Method according to any one of claims 14-17, wherein the compound is an siRNA comprising a sense strand of 17-23 nucleotides homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 or 2, and an antisense strand of 17-23 nucleotides complementary to the sense strand.

19. Method according to claim 18, wherein the siRNA further comprises a loop region connecting the sense and the antisense strand.

20. Method according to claim 19, wherein the loop region comprises the nucleic acid of SEQ ID NO: 171.

21. Method according to any one of claims 1-20, wherein the compound is modified to confirm resistance to nucleolytic degradation or to enhance the activity, cellular distribution, or cellular uptake.

22. Method according to claim 21, wherein the modification comprises a modified internucleoside linkage, a modified nucleic acid base, a modified sugar, and/or a chemical linkage of the oligonucleotide to one or more moieties or conjugates.

23. An isolated nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 2 and 5-170, or a functional derivative or fragment thereof, or an isolated nucleic acid sequence complementary thereto.

24. An isolated nucleic acid sequence according to claim 23, wherein the nucleic acid sequence is further modified to confirm resistance to nucleolytic degradation or to enhance the activity, cellular distribution, or cellular uptake.

25. An isolated nucleic acid sequence according to claim 24, wherein the modification comprises a modified internucleoside linkage, a modified nucleic acid base, a modified sugar and/or a chemical linkage of the oligonucleotide to one or more moieties or conjugates.

26. An isolated nucleic acid sequence according to any one of claims 23-25 for use as a medicament.

27. An isolated nucleic acid according to claim 29, wherein said use is in the treatment of a pathological condition involving cognitive impairment or a susceptibility to the condition, such as Alzheimer's disease.

28. An siRNA molecule comprising a sense strand of 17-23 nucleotides homologous to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 or 2, and an antisense strand of 17-23 nucleotides complementary to the sense strand.

29. An siRNA according to claim 28, further comprising a loop region connecting the sense and the antisense strand.

30. An siRNA molecule according to claim 29, wherein the loop region comprises the nucleic acid sequence of SEQ ID NO: 171.

31. A vector comprising a nucleic acid sequence according to any one of claims 23-25.

32. A vector according to claim 31, wherein said vector is selected from the group consisting of an adenovirus, a retrovirus, an alphavirus, a lentivirus, an adeno-associated virus, a herpes simplex virus and a sendai virus.

33. A pharmaceutical composition comprising a nucleic acid sequence according to any one of claims 23-25, and a pharmaceutically acceptable solvent, diluent, excipient and/or carrier.

34. Method for treatment, prevention or amelioration of a pathological condition involving cognitive impairment or a susceptibility to the condition in a mammalian subject, said method comprising the step of administering a pharmaceutical composition according to claim 33.

35. Method of reducing the level of amyloid-beta peptide 11-42 or 11-40, 1-42 or 1-40 in a subject, said method comprising the step of administering a pharmaceutical composition according to claim 33.

36. Method for diagnosis of a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject, said method comprising the steps of:

(a) determining the nucleic acid sequence of at least one of the genes of SEQ ID NO: 1 or 2 within the genomic DNA of said subject;

(b) comparing the sequence from step (a) with the nucleic acid sequence obtained from a database and/or a healthy subject; and

(c) identifying any difference(s) related to the onset of the pathological condition.

37. Method for diagnosis of a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject, said method comprising the steps of:

(a) determining the amount of polypeptide of SEQ ID NO: 3 or 4 in a sample from said subject; and

(b) comparing the amount determined in step (a) with the amount of said polypeptide is a sample from a healthy individual;

wherein the increase in the sample of said subject as compared to the healthy individual is indicative for the onset or presence of said pathological condition.

38. Method according to claim 36 or 37, wherein the pathological condition is Alzheimer's disease.

39. Use of a compound that inhibits the activity and/or the expression of a polypeptide according to SEQ ID NO:1 or 2, in the manufacture of a medicament for the treatment of Alzheimer's Disease.

40. Use according to claim 39, wherein said compound is selected from the group consisting of: SHU9119, an Agouti peptide or protein, an Agouti-related peptide, Ro 27-3225, Ro 27-4680, HS028, HS024, HS014, HS131, and any of the compounds as described in US 6,699,873, US 6,350,430 and US 6,693,165.

41. Use according to claim 39, wherein said compound is identified according to any one of the methods of claims 1-22.

42. Use according to claim 39, wherein said compound is any one of the isolated nucleic acid sequences of claims 23-25.

43. Use according to claim 39, wherein said compound is any one of the siRNA's of claims 28-30.

Figure 1

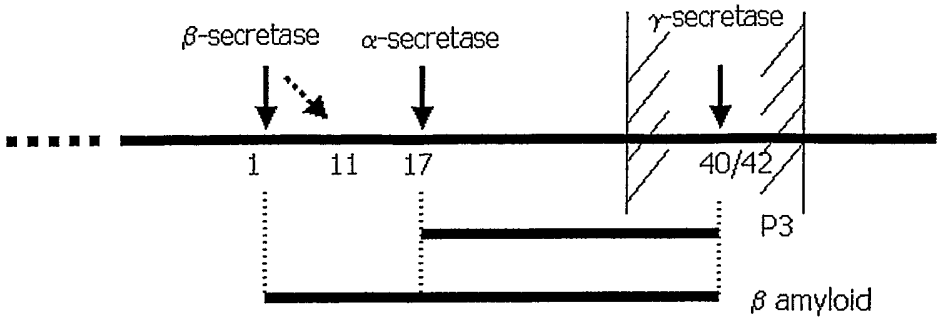
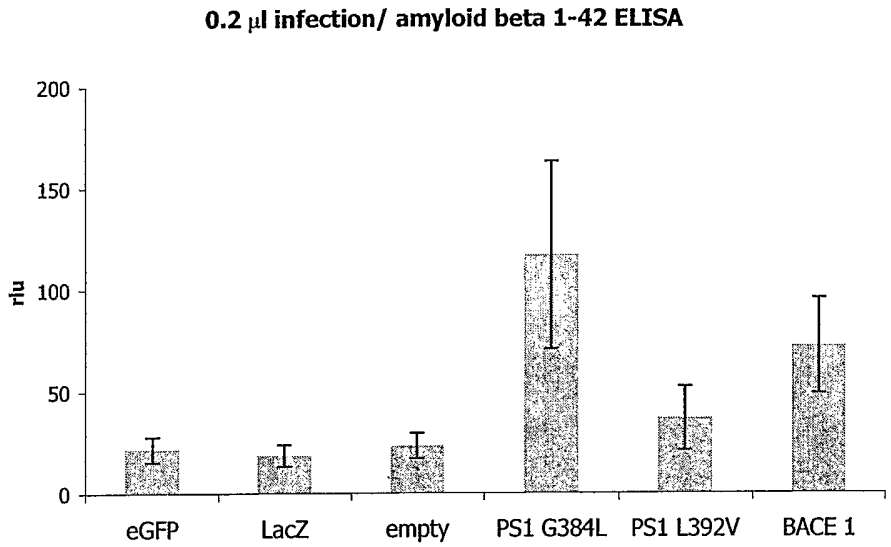


Figure 2


0.2 µl infection/ amyloid beta 1-42 ELISA
rlu
200
150
100
50
0
eGFP
LacZ
empty
PS1 G384L
PS1 L392V
BACE 1

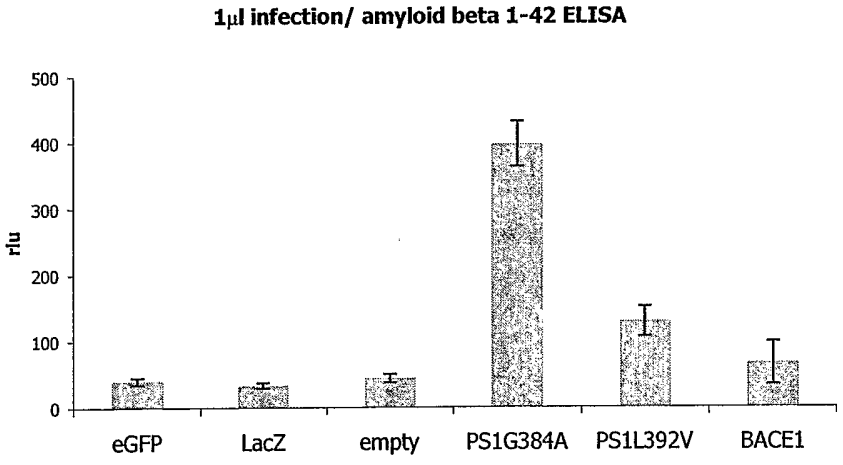
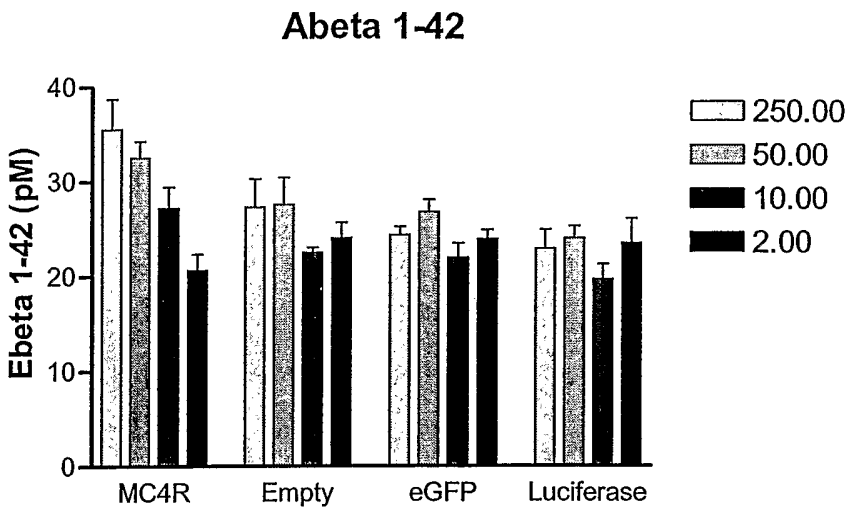

1µl infection/ amyloid beta 1-42 ELISA
rlu
500
400
300
200
100
0
eGFP
LacZ
empty
PS1G384A
PS1L392V
BACE1

Figure 3

Panel A



Panel B

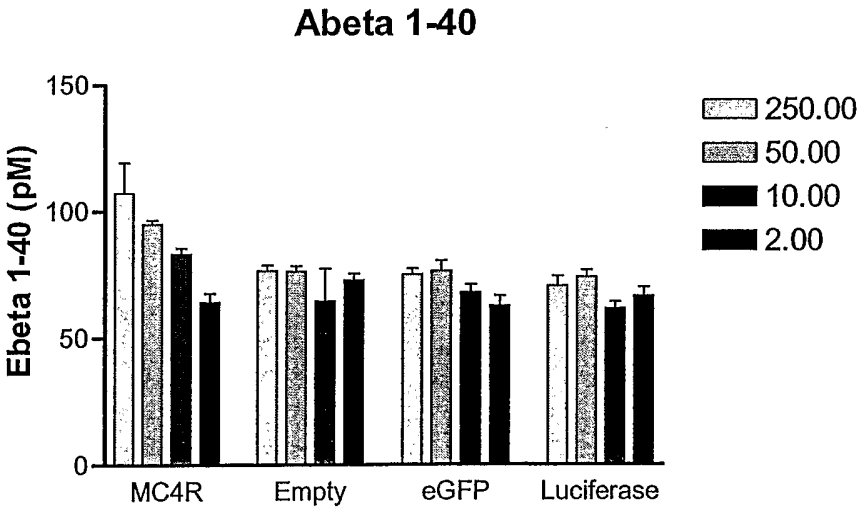
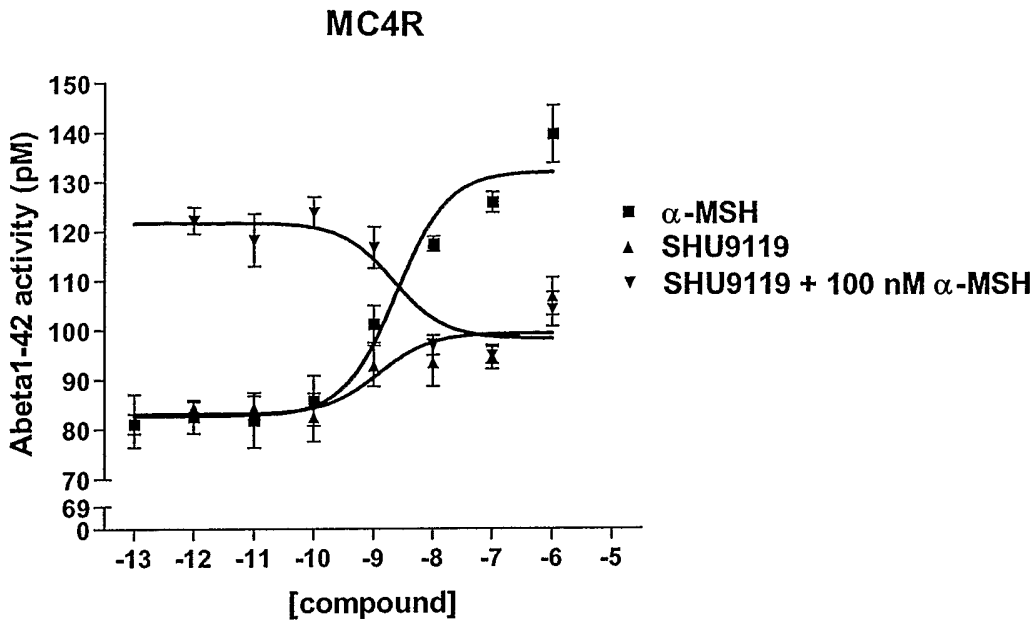


Figure 4

Panel A



Panel B

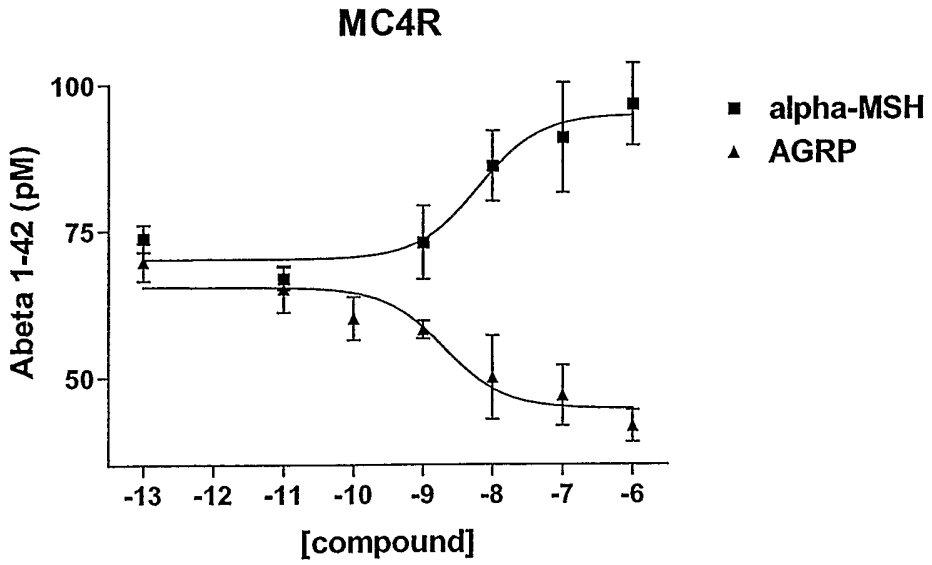


Figure 5

Sequence alignment:

Algorithm: ClustalW
Substitution matrix: *Gonnet 250*
Gap open penalty: 10
Gap extension penalty: 0.05

```
MC4R            ------------MVNSTHRGMHTSLHLWNRSSYRLHSNASESLG---------------- 32
MC3R            MSIQKTYLEGDFVFPVSSSSFLRTLLEPQLGSALLTAMNASCCLPSVQPTLPNGSEHLQA 60
                             :  *:   ::* *.    *:     *** .*

MC4R            ---KGYSDGGCYEQLFVSPEVFVTLGVISLLENILVIVAIAKNKNLHSPMYFFICSLAVA 89
MC3R            PFFSNQSSSAFCEQVFIKPEVFLSLGIVSLLENILVILAVVRNGNLHSPMYFFLCSLAVA 120
                   .. *...  **:*:.****::**::*********:*:.:* *********:******

MC4R            DMLVSVSNGSETIIITLLNST-DTDAQSFTVNIDNVIDSVICSSLLASICSLLSIAVDRY 148
MC3R            DMLVSVSNALETIMIAIVHSDYLTFEDQFIQHMDNIFDSMICISLVASICNLLAIAVDRY 180
                ********. ***:*::::*   *  :.*  ::**::**:** **:****.**:******

MC4R            FTIFYALQYHNIMTVKRVGIIISCIWAACTVSGILFIIYSDSSAVIICLITMFFTMLALM 208
MC3R            VTIFYALRYHSIMTVRKALTLIVAIWVCCGVCGVVFIVYSESKMVIVCLITMFFAMMLLM 240
                .******:**.****::.  :* .**..* *.*::**:**:*. **:*******:*: **

MC4R            ASLYVHMFLMARLHIKRIAVLPGTG--AIRQGANMKGAITLTILIGVFVVCWAPFFLHLI 266
MC3R            GTLYVHMFLFARLHVKRIAALPPADGVAPQQHSCMKGAVTITILLGVFIFCWAPFFLHLV 300
                .:*******:****:****.** :.  * :* : ****:*:***:***:.*********:

MC4R            FYISCPQNPYCVCFMSHFNLYLILIMCNSIIDPLIYALRSQELRKTFKEIICCYPLGGLC 326
MC3R            LIITCPTNPYCICYTAHFNTYLVLIMCNSVIDPLIYAFRSLELRNTFREILCGCNGMNLG 360
                : *:** ****:*: :*** **:******:*******:** ***:**:**:*     .*

MC4R            DLSSRY 332
MC3R            ------
                . ::
```

CONSENSUS SYMBOLS:
An alignment will display by default the following symbols denoting the degree of conservation observed in each column:
"*" means that the residues or nucleotides in that column are identical in all sequences in the alignment.
":" means that conserved substitutions have been observed, according to the following groups: AVFPMILW, DE, RHK or STYHCNGQ.
"." means that semi-conserved substitutions are observed.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/005624

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/50 C07K14/00 C12N15/12 C12Q1/68 G01N33/68
A61K48/00 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 287 763 B1 (LEE FRANK ET AL) 11 September 2001 (2001-09-11) Seq Id No 2, col. 3, line 15 - col. 4, line 32col. 9, line 55 - col. 13, line 35, col. 22, line 57 - col. 30, line 29, col. 46, line 38 - col. 9, line 10 ----- -/--	3,4,6-33

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2005	12. 07. 2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hoesel, H

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/005624

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GANTZ I ET AL: "MOLECULAR CLONING EXPRESSION AND GENE LOCALIZATION OF A FOURTH MELANOCORTIN RECEPTOR" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 268, no. 20, 15 July 1993 (1993-07-15), pages 15174-15179, XP002051983 ISSN: 0021-9258 p. 15174/15175 "Receptor Expression", Fig. 1	23,31
A	GALIMBERTI D ET AL: "ALPHA-MSH PEPTIDES INHIBIT PRODUCTION OF NITRIC OXIDE AND TUMOR NECROSIS FACTOR-ALPHA BY MICROGLIAL CELLS ACTIVATED WITH BETA-AMYLOID AND INTERFERON GAMMA" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 263, 16 September 1999 (1999-09-16), pages 251-256, XP000941934 ISSN: 0006-291X abstract, p. 255, col. 2, lines 31 - 43	1-43
A	BUTLER A A ET AL: "THE MELANOCORTIN RECEPTORS: LESSONS FROM KNOCKOUT MODELS" NEUROPEPTIDES, vol. 36, no. 2/3, April 2002 (2002-04), pages 77-84, XP008012345 ISSN: 0143-4179 abstract, Tab. 1, p. 79, col. 2, line 15 - p. 82, col,2, line 23	1-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/005624

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 34 and 35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-3, 5-43 (all in part);4

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3,5-43 (all in part); 4

method for identifying a compound that influences the processing of amyloid beta protein in a cell based assay, wherein the cell expresses melanocortin receptor 4 ("Mc4R"); nucleic acids encoding Mc4R or derived from Mc4R coding sequence, methods for diagnosing Alzheimer's disease or a predisposition thereto based on detecting Mc4R protein or nucleic acid, use of Mc4R antagonists in the treatment of Alzheimer's disease

---

2. claims: 1-3,5-43 (all in part.)

method for identifying a compound that influences the processing of amyloid beta protein in a cell based assay, wherein the cell expresses melanocortin receptor 3 ("MC3R"); nucleic acids encoding Mc3R or derived from Mc3R coding sequence, methods for diagnosing Alzheimer's disease or a predisposition thereto based on detecting Mc3R protein or nucleic acid, use of Mc3R antagonists in the treatment of Alzheimer's disease

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2004/005624

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6287763 B1	11-09-2001	US 5932779 A	03-08-1999
		US 5908609 A	01-06-1999
		CN 1227496 A	01-09-1999
		EP 0915706 A1	19-05-1999
		JP 2002514041 T	14-05-2002
		AU 723135 B2	17-08-2000
		AU 3383697 A	07-01-1998
		BR 9709684 A	09-05-2000
		CA 2257857 A1	18-12-1997
		KR 2000034781 A	26-06-2000
		TR 9802572 T2	22-03-1999
		WO 9747316 A1	18-12-1997