β-Adrenergic Receptors and G Protein-Coupled Receptor Kinase-2 in Alzheimer's Disease: A New Paradigm for Prognosis...

Article *in* Journal of Alzheimer's disease: JAD · December 2012

DOI: 10.3233/JAD-121813 · Source: PubMed

CITATIONS
25

READS
93

12 authors, including:

Klara Komici

17 PUBLICATIONS **128** CITATIONS

SEE PROFILE

Pasquale Perrone-Filardi

University of Naples Federico II

192 PUBLICATIONS **6,276** CITATIONS

SEE PROFILE

Nicola Ferrara

University of Naples Federico II

280 PUBLICATIONS **4,264** CITATIONS

SEE PROFILE

Dario Leosco

University of Naples Federico II

176 PUBLICATIONS **2,284** CITATIONS

SEE PROFILE

Journal of Alzheimer's Disease 34 (2013) 341–347
DOI 10.3233/JAD-121813
IOS Press

Review

β-Adrenergic Receptors and G Protein-Coupled Receptor Kinase-2 in Alzheimer's Disease: A New Paradigm for Prognosis and Therapy?

Grazia Daniela Femminella[a,1], Giuseppe Rengo[a,b,1,*], Gennaro Pagano[a], Claudio de Lucia[a], Klara Komici[a], Valentina Parisi[a], Alessandro Cannavo[a], Daniela Liccardo[a], Carlo Vigorito[a], Pasquale Perrone Filardi[c], Nicola Ferrara[a] and Dario Leosco[a]
[a]*Department of Medical Translational Sciences, Federico II University of Naples, Naples, Italy*
[b]*"Salvatore Maugeri" Foundation – IRCCS – Institute of Telese Terme (BN), Italy*
[c]*Department of Advanced Biomedical Sciences, Federico II University of Naples, Naples, Italy*

Accepted 31 October 2012

Abstract. Alzheimer's disease (AD) is a devastating form of dementia that imposes a severe burden on health systems and society. Although several aspects of AD pathogenesis have been elucidated over the last few decades, many questions still need to be addressed. In fact, currently available medications only provide symptomatic improvement in patients with AD without affecting disease progression. The β-adrenergic receptor (β-AR) system can be considered a possible target that deserves further exploration in AD. The central noradrenergic system undergoes substantial changes in the course of AD and β-ARs have been implicated not only in amyloid formation in AD brain but also in amyloid-induced neurotoxicity. Moreover, clinical evidence suggests a protective role of β-AR blockers on AD onset. In addition to that, post-receptor components of β-AR signaling seem to have a role in AD pathogenesis. In particular, the G protein coupled receptor kinase 2, responsible for β-AR desensitization and downregulation, mediates amyloid-induced β-AR dysfunction in neurons, and its levels in circulating lymphocytes of AD patients are increased and inversely correlated with patient's cognitive status. Therefore, there is an urgent need to gain further insight on the role of the adrenergic system components in AD pathogenesis in order to translate preclinical and clinical knowledge to more efficacious prognostic and therapeutic strategies.

Keywords: Alzheimer's disease, amyloid, beta-adrenergic receptors, G-protein coupled receptor kinase-2

Alzheimer's disease (AD) is the most common form of age-related dementia, affecting more than 35 million people in the world and about 4.4% of the elderly population [1]. It is a progressive neurodegenerative disorder characterized by various mental dysfunctions including cognitive impairment, emotional instability, and changes in personality [2]. In advanced disease, diffuse cerebral atrophy with widened sulci and ventricles enlargement are observed. Microscopically, loss of selected populations of neurons occurs. Neuronal degeneration in AD affects different subcortical nuclei: nucleus basalis of Meynert, the major source of the neurotransmitter acetylcholine; locus coeruleus (LC)

[1]These authors contributed equally to this work.

*Correspondence to: Dr. Giuseppe Rengo, MD, PhD, "Salvatore Maugeri" Foundation – IRCCS – Institute of Telese Terme (BN), Via Bagni Vecchi 1, 82037 Telese Terme (BN), Italy. Tel./Fax: +39 0817462267; E-mail: giuseppe.rengo@unina.it.

ISSN 1387-2877/13/$27.50

which produces norepinephrine; substantia nigra pars compacta, a source of dopamine; and dorsal raphe nucleus, which releases serotonin. Each of these nuclei projects to wide areas of cerebral cortex or basal ganglia, thus integrating inputs from different sources and modulating several physiological processes (arousal and sleep, blood pressure, attention and vigilance, memory and learning, mood and aggression).

Several molecular lesions have been detected in AD. Among them, the deposition of amyloid plaques, which are largely composed of amyloid-β (Aβ), is a major characteristic of AD neuropathology and considered to be the primary cause of the disease. Aβ is generated from the amyloid-β protein precursor (AβPP) via sequential cleavages by β- and γ-secretases. The γ-secretase is pivotal, because mutations in the catalytic subunit presenilin-1 account for most cases of familial AD [3].

Multiple lines of evidence suggest that, either resulting from an inherited genetic mutation or caused by an environmental insult, over-accumulation of Aβ in AD brain is one of the common points in which multiple initiating pathways may converge.

Moreover, neurodegenerative diseases such as AD are associated with microvascular dysfunction, neurovascular degeneration, and alterations in blood-brain barrier function. Such deficits impair the clearance of neurotoxic molecules, such as Aβ, that accumulate in brain and reduce the brain's supply of oxygen and nutrients. Thus, vascular dysfunction is tightly linked to neuronal dysfunction in AD [4].

However, despite the multiple hypothesis on AD pathogenesis that can contribute to the onset and the progression of the disease, the current pharmacological treatment is mainly based on drugs that increase brain acetylcholine levels, according to evidence derived from studies on the nucleus basalis neuronal loss in AD. In any case, these molecules induce only a temporary and symptomatic improvement in patients with AD [5]. Thus, there is an increasing interest in exploring new therapeutic targets in AD. In this vein, over the last few decades, the cerebral adrenergic system has emerged as a potential candidate for further investigation in AD.

ADRENERGIC SYSTEM IN ALZHEIMER'S DISEASE

It is well established that LC is the main norepinephrine source in the central nervous system (CNS), providing an extensive network of neuronal projections to all major brain regions, including the neocortex and hippocampus [6]. Several studies have indicated that LC degeneration might have a role in AD pathogenesis. In fact, numerous postmortem studies on AD brains have documented an early LC degeneration during the course of AD [7, 8]. Moreover, other studies have observed that LC neuronal loss in AD patients is paralleled by a significant reduction in cortical norepinephrine concentration that is correlated with cognitive impairment [9], while others have demonstrated that in the LC of AD subjects, a significant decrease in the norepinephrine transporter is observed, and this decrease correlates with the progression of the disease [10]. However, other studies reported an increased norepinephrine concentration in cerebrospinal fluid [11], suggesting that in AD brain, there is a compensation occurring in the remaining noradrenergic neurons [12]. In addition, it has been observed that degeneration of LC neurons can occur in patients with mild cognitive impairment [13]. Moreover, along with its role as a neurotransmitter, norepinephrine has also been recently described to exert anti-inflammatory actions within the CNS, since the decrease of norepinephrine in LC projection areas has been shown to facilitate the inflammatory reaction of microglial cells in AD [6] and to exacerbate neuroinflammation induced by the deposition of amyloid peptides [14].

β-ADRENERGIC RECEPTORS (β-AR) AND ALZHEIMER'S DISEASE

The effects of norepinephrine are mediated by the modulation of AR in brain, with both α- and β-AR playing an important role in brain neurotransmission. Functional studies of AR in human brain have focused mainly on α_2-receptors regulating neurotransmitter release [15, 16], although all three known subtypes of β-ARs (β_1-AR, β_2-AR, and β_3-AR) are found in brain, with the β_3-AR subtype having limited expression [17]. Some imaging studies have documented that β-AR are located in the cerebral cortex, nucleus accumbens, and striatum. At lower density, they are also present in amygdala, hippocampus, and cerebellum. In particular, β_2-AR have a role in the regulation of glial proliferation during ontogenic development, after trauma, and in neurodegenerative diseases, while β_1-AR density is affected by stress, in several mood disorders (depression, schizophrenia) and during treatment with antidepressants [18]. Moreover, it has been demonstrated that both β_1- and β_2-ARs are present on rat microglial cells [19].

β-ARs are members of the large family of seven membrane-spanning, G-protein-coupled receptors (GPCR). Although they respond to the same ligands (norepinephrine and epinephrine) β-ARs differ significantly in the types of cellular responses they mediate [20, 21]. Their agonist-mediated activation catalyzes the exchange of GTP for GDP on the Gα-subunit of G proteins, resulting in the dissociation of the heterotrimer into active Gα- and Gβγ-subunits, which are competent to signal independently. The activated Gα-subunit dissociates from the G protein complex and stimulates (Gs) or inhibits (Gi) adenylyl cyclase, and therefore modulates the intracellular amount of cyclic AMP (cAMP) [22].

Moreover, the dissociation of the Gβγ subunit is able to facilitate the juxtaposition of AR and G-protein receptor kinases (GRKs), which ultimately mediate the phosphorylation of AR [23]. GRK-mediated phosphorylation results in agonist-dependent desensitization (homologous desensitization) of ARs and promotes the binding of the cytosolic proteins arrestins. Once bound to the receptor, arrestins interdict further G-protein coupling and target the activated receptor for endocytosis (downregulation) [24].

Over the last few decades, there has been growing interest in exploring the potential implications of β-ARs in the pathogenesis of AD. In the late 1980 s, Kalaria and colleagues found that in AD subjects selective changes in the number of β-ARs occurred in different brain regions and these changes were paralleled by reduction in the number of LC cells and norepinephrine concentrations in putamen and frontal cortex [25]. Importantly, β-ARs have also been implicated in Aβ formation in AD. In fact, it has been shown that stimulation of AR by norepinephrine, coupled with increased cAMP formation, resulted in AβPP overexpression in rat astrocytes. Interestingly, the increase in AβPP caused by norepinephrine treatment was inhibited by the β-AR antagonist propranolol [26]. In 2006, Igbavboa et al. demonstrated that Aβ stimulated cAMP formation, and this stimulation was inhibited by selective β-AR antagonists in mouse primary cortical astrocytes. Furthermore, this effect of Aβ on cAMP levels was associated with an increase in apolipoprotein E levels which in turn was inhibited by selective β-AR antagonists. The authors suggest that a potential association between β-AR and apolipoprotein E homeostasis could be an important mechanism in counteracting Aβ-induced neurotoxicity in astrocytes [27]. Further insight on the important role of β-AR in amyloid formation came from a study by Ni and colleagues. In particular, they demonstrated that the activation of β_2-AR increases Aβ production *in vitro* and amyloid plaque formation *in vivo* by enhancing γ-secretase activity. The mechanism proposed in this study involved the association of β_2-AR with presenilin-1 and required agonist-induced endocytosis of β_2-AR. Moreover, chronic treatment with β_2-AR agonists increased cerebral amyloid plaques, while the β_2-AR-selective antagonist ICI 118,551 ameliorated amyloid plaque pathology in mouse models of AD, suggesting that abnormal β_2-AR activation might contribute to Aβ accumulation in AD pathogenesis (Fig. 1) [28]. Similar results were obtained in a mouse model of acute restraint stress, in which administration of the β_2-AR selective agonist clenbuterol enhanced the stress-induced Aβ peptides production, while the β_2-AR-selective antagonist ICI 118,551 reduced it [29]. More recently, Wang and colleagues demonstrated that in primary cortical neurons, Aβ is able to bind to β_2-AR and to induce receptors internalization and degradation. This process is prevented by pretreatment with β-AR antagonist timolol and is dependent on GRK2-mediated receptor phosphorylation [30]. In addition, genetic studies have shown a correlation between β-AR polymorphisms and AD. In particular, Yu and colleagues investigated the association of two polymorphisms in the β_2-AR gene (Gly16Arg and Gln27Glu) with the risk of late onset AD in a Chinese population. Their results indicate that both β_2-AR gene variants were associated with an increased risk of late onset AD, and they also showed a significant correlation with the apolipoprotein E ε4 allele, suggesting that the Gly16 and Gln27 variants are associated with the development of AD, and might also interact with apolipoprotein E ε4 status [31].

Additional evidence on the importance of the adrenergic system in AD came from studies on AR blockers. In particular, it has been demonstrated that carvedilol, a nonselective α- and β-AR blocker, significantly reduced oligomerization and deposition of Aβ peptides in the cerebral cortex and hippocampus of transgenic murine model of AD [32]. The same authors also showed that carvedilol is able to increase basal synaptic transmission and long term potentiation in AD mice, thus improving neuronal plasticity [33]. Further data emerged from clinical studies examining the association of cardiovascular medication use and AD incidence. Interestingly, in the Dementia Progression Study of the Cache County Study, Rosenberg demonstrated that use of statins and β-blockers was associated with a slower annual rate of functional decline in this AD population, while other cardiovascular drugs did not affect the functional decline [34].

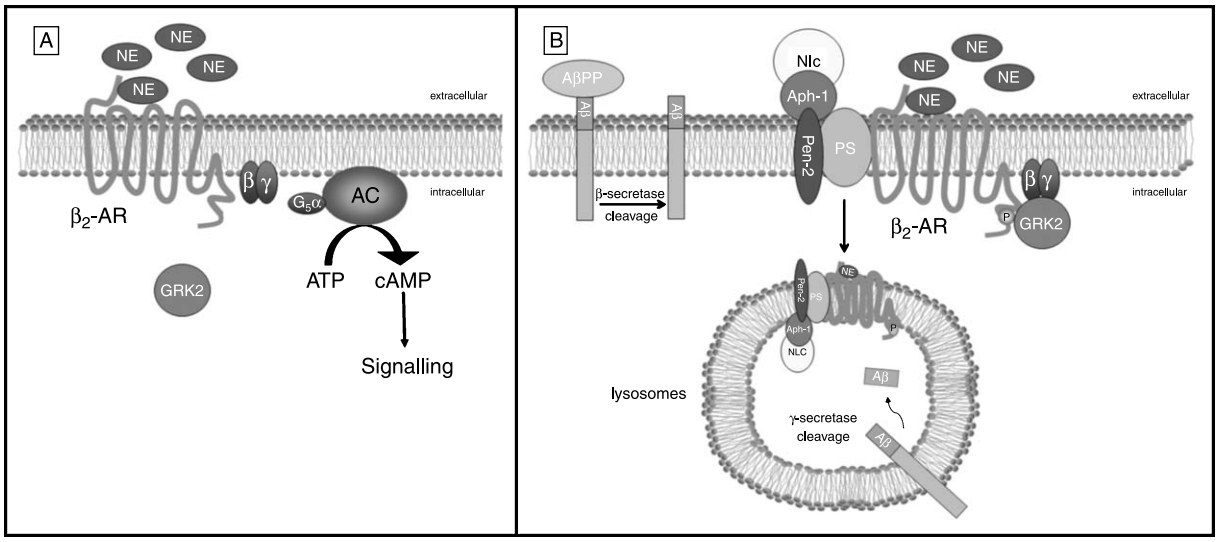

Fig. 1. Mechanisms of action for β_2 adrenergic receptor (β_2-AR) and G-protein-coupled receptor kinase 2 (GRK2) in amyloid-β (Aβ) production as proposed by Ni et al. [28]. A) G-protein-dependent β_2-AR signaling pathway. Receptor activation by norepinephrine (NE) leads to stimulation of adenylate cyclase (AC) and elevated cAMP. B) Agonist-bound β_2-AR phosphorylation by GRK2 induces endocytosis of the β_2-AR/γ-secretase complex. In lysosomes γ-secretase activity is increased, leading to increased production of Aβ. AβPP, amyloid-β protein precursor; PS, presenilin; Nic, nicastrin; Pen-2, presenilin enhancer 2; Aph-1, anterior pharynx-defective 1.

Despite the fact that the evidence currently available is in some cases conflicting, taken together, the findings discussed above suggest that β-ARs might represent a novel target in AD treatment that deserves further exploration and whose role needs definite clarification.

GRK2 AND ALZHEIMER'S DISEASE

Besides ARs alterations, several lines of evidence have suggested that there is a widespread dysfunction in post-receptor components of GPCR signaling in AD brain, occurring at the receptor-G protein interface that is the site of action of GRKs [35].

The GRKs are a family of cytosolic serine/threonine kinases consisting of seven isoforms that share structural and functional similarities. Their primary function is to desensitize activated GPCRs and target them for internalization. While some GRKs are expressed in a wide variety of tissues, others display a more restricted expression pattern [24].

Recent studies on AD pathogenetic mechanisms have primarily focused on the possible involvement of GRK2 and GRK5 in GPCR-G protein interaction dysfunction of the disease.

As for GRK5, different studies have demonstrated that in AD brain, Aβ deposition can lead to reduced membrane GRK5 levels. The so-called "GRK5 deficiency" impairs muscarinic receptors regulation, ultimately leading to reduced acetylcholine release and neuronal degeneration [36].

GRK2, also known as β-AR kinase 1 (βARK1), is the most abundant GRK in the heart, and its levels are increased in different cardiovascular diseases associated with impaired cardiac function [37–42]. In brain, GRK2 is widely distributed, as it is found in both postsynaptic densities and presynaptic axon terminals consistent with a general role of GRK2 in the desensitization of synaptic receptors [43].

The first to examine the possible involvement of GRK2 in AD pathology were Suo and colleagues. These authors evaluated the molecular mechanisms underlying the effects of soluble Aβ in murine microglial cells and in an AD transgenic mouse model. They discovered that soluble Aβ can potentiate, in a dose-dependent manner, microglial release of inflammatory cytokines and, more importantly, they also found that Aβ treatment of microglial cells *in vitro* decreased membrane-bound levels of GRK2 and GRK5, leading to cytosolic GRK accumulation. This process resulted in retarded GPCR desensitization and prolonged GPCR signaling, leading to receptors hyperactivity. Analysis of early-onset AD transgenic mice revealed that while total brain GRK2 levels increased, the kinase in the membrane fraction was significantly reduced. Furthermore, the GRK abnormality observed

in vivo was associated with the very early increase of brain soluble Aβ levels and it occurred before the onset of cognitive decline. These observations imply that the GRK abnormality may be an early pathogenetic event manifesting at prodromic and early stages of AD that is closely associated with very early accumulation of brain soluble Aβ [44]. Another report demonstrated that GRK2 colocalized with neurofibrillary tangles in AD mixed with Lewy body disease and AD human brains, but was not present in Lewy bodies [45].

An important aspect of GPCR signaling is that system properties in circulating white blood cells appear to mirror those observed in solid tissues. In this regard, our group showed that lymphocyte GRK2 levels are well correlated to cardiac protein concentrations in patients with heart failure [46].

According to this concept, we examined for the first time the role of lymphocyte GRK2 in patients with AD. In our study, we evaluated GRK2 mRNA levels and protein expression in peripheral blood lymphocytes of AD patients with mild or moderate/severe cognitive impairment and in age-matched healthy subjects. We found that both GRK2 mRNA and protein expression were significantly higher in AD patients lymphocytes compared to controls. Furthermore, lymphocyte GRK2 levels were significantly correlated to the degree of cognitive decline. Importantly, these data suggest that lymphocyte GRK2 could emerge as a novel biological marker of this syndrome [47].

As discusses above, increasing evidence strongly support the hypothesis that vascular damage is an early contributor to the development of AD. In fact, vascular injury and parenchymal inflammation perpetuate protein aggregation and oxidation in brain. Moreover, AD and vascular dementia have several risk factors in common, including hypertension, aging, and dyslipidemia, and it is well established that cardiovascular risk factors are in part responsible for the neurovascular unit dysfunction observed both in AD and vascular dementia [48]. Based on this hypothesis, Obrenovich and colleagues explored, by morphological studies, cellular and subcellular localization of GRK2 in the early phases of AD and in ischemia-reperfusion injury models of chronic brain hypoperfusion, that mimic mild cognitive impairment and vascular changes in AD pathology. They demonstrated the early overexpression of GRK2 in the cerebrovasculature, particularly in endothelial cells following chronic hypoperfusion. They also found a significant increase in GRK2 immunoreactivity in endothelial cells of AD patients, which preceded any amyloid deposition. Thus they propose to consider AD and AD-like pathology as disorders of the cerebrovasculature and to use GRK2 as an early marker of cerebrovascular aging complications in age-associated diseases involving cerebrovascular abnormalities, neurodegeneration, and cognitive impairment before any amyloid deposition can be seen [49].

FUTURE PERSPECTIVES AND THERAPEUTIC OPPORTUNITIES

Besides established treatment for AD and novel therapeutic strategies currently pursued, such as secretase-modulating drugs or Aβ-targeted immunotherapy [50], several pathogenic mechanisms involved in AD still need to be clarified.

In this vein, β-AR and GRKs are two possible targets that deserve further exploration in this field. AR system and its regulation by GRK have a well-established role in cardiovascular diseases and there are numerous parallels that can be drawn between neurodegenerative and cerebrovascular disorders [4, 48]. In the last decades, in fact, the "neuroncentric" hypothesis for AD pathogenesis has gradually left space to the neurovascular hypothesis, in which β-ARs and GRK2 could certainly play a role that deserves further insight. Studies on the use of β-blockers have shown that these drugs are able to slow cognitive decline in AD patients but the underlying mechanism of action has not been explored, neither the effects of β-blockers on brain GRK2 levels [34]. As for GRK2 modulation in AD, evidence available have shown that GRK2 inhibition, via the βARKct peptide, is able to block the Aβ-induced β_2AR internalization *in vitro*, but the effects of GRK2 on Aβ accumulation have not yet been investigated [30]. Particularly intriguing is the finding that GRK2 is associated with vascular damage in AD [49]. This latter evidence suggests that GRK2 involvement in AD could also be Aβ-independent, thus representing an alternative therapeutic target in the disease. Moreover, a recent study identified the selective serotonin reuptake inhibitor paroxetine as a direct inhibitor of GRK2 activity [51]. If this kinase emerged as a key factor involved in AD pathogenesis, then the possibility to use selective drugs as paroxetine to modulate its expression would have a tremendous therapeutic impact. In addition, lymphocyte GRK2 levels have been shown to correlate with cognitive status of AD patients [47], thus peripheral levels of the kinase could be used as an easily accessible biomarker to monitor patients' cognitive function and response to specific therapies.

CONCLUSIONS

AD is a major and growing public health problem and medications currently available are symptomatic drugs that do not significantly affect the progression of the disease. Hence, there is an urgent medical need for the development of new therapeutic strategies targeting different pathogenic mechanisms involved in AD onset and progression. In this view, the β-AR system emerges as a novel and interesting player in the pathogenesis of AD, for its involvement both in the neuronal/glial alterations and vascular modifications occurring in AD. Moreover, the availability of several molecules acting on β-ARs, together with long-term experience with their use, makes β-AR signaling and GRK modulation valuable targets for AD treatment. Nevertheless, additional preclinical and clinical studies are required to clearly assess the role of β-AR system-modulating drugs as feasible tools for AD therapy.

DISCLOSURE STATEMENT

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=1564).

REFERENCES

[1] Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, Arrighi HM (2012) Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/4) among patients diagnosed with Alzheimer's disease: A systematic review and meta-analysis. *Neuroepidemiology* **38**, 1-17.

[2] Yu JT, Wang ND, Ma T, Jiang H, Guan J, Tan L (2011) Roles of β-adrenergic receptors in Alzheimer's disease: Implications for novel therapeutics. *Brain Res Bull* **84**, 111-117.

[3] Querfurth HW, LaFerla FM (2010) Alzheimer's disease. *N Engl J Med* **362**, 329-344.

[4] Zlokovic BV (2011) Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* **12**, 723-738.

[5] Lyness SA, Zarow C, Chui HC (2003) Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: A meta-analysis. *Neurobiol Aging* **24**, 1-23.

[6] Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A, Dumitrescu-Ozimek L, Terwel D, Jardanhazi-Kurutz D, Walter J, Kirchhoff F, Hanisch UK, Kummer MP (2010) Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. *Proc Natl Acad Sci U S A* **107**, 6058-6063.

[7] Zarow C, Lyness SA, Mortimer JA, Chui HC (2003) Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch Neurol* **60**, 337-341.

[8] Haglund M, Sjöbeck M, Englund E (2006) Locus ceruleus degeneration is ubiquitous in Alzheimer's disease: Possible implications for diagnosis and treatment. *Neuropathology* **26**, 528-532.

[9] Matthews KL, Chen CP, Esiri MM, Keene J, Minger SL, Francis PT (2002) Noradrenergic changes, aggressive behavior, and cognition in patients with dementia. *Biol Psychiatry* **51**, 407-416.

[10] Gulyás B, Brockschnieder D, Nag S, Pavlova E, Kása P, Beliczai Z, Lágrádi A, Gulya K, Thiele A, Dyrks T, Halldin C (2010) The norepinephrine transporter (NET) radioligand (S,S)-[18F]FMeNER-D2 shows significant decreases in NET density in the human brain in Alzheimer's disease: A post-mortem autoradiographic study. *Neurochem Int* **56**, 789-798.

[11] Raskind MA, Peskind ER, Holmes C, Goldstein DS (1999) Patterns of cerebrospinal fluid catechols support increased central noradrenergic responsiveness in aging and Alzheimer's disease. *Biol Psychiatry* **46**, 756-765.

[12] Szot P, White SS, Greenup JL, Leverenz JB, Peskind ER, Raskind MA (2006) Compensatory changes in the noradrenergic nervous system in the locus ceruleus and hippocampus of postmortem subjects with Alzheimer's disease and dementia with Lewy bodies. *J Neurosci* **26**, 467-478.

[13] Grudzien A, Shaw P, Weintraub S, Bigio E, Mash DC, Mesulam MM (2007) Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. *Neurobiol Aging* **28**, 327-335.

[14] Jardanhazi-Kurutz D, Kummer MP, Terwel D, Vogel K, Thiele A, Heneka MT (2011) Distinct adrenergic system changes and neuroinflammation in response to induced locus ceruleus degeneration in APP/PS1 transgenic mice. *Neuroscience* **176**, 396-407.

[15] Raiteri M (2006) Functional pharmacology in human brain. *Pharmacol Rev* **58**, 162-193.

[16] Lymperopoulos A, Rengo G, Koch WJ (2007) Adrenal adrenoceptors in heart failure: Fine-tuning cardiac stimulation. *Trends Mol Med* **13**, 503-511.

[17] Rainbow TC, Parsons B, Wolfe BB (1984) Quantitative autoradiography of beta 1- and beta 2-adrenergic receptors in rat brain. *Proc Natl Acad Sci U S A* **81**, 1585-1589.

[18] van Waarde A, Vaalburg W, Doze P, Bosker FJ, Elsinga PH (2004) PET imaging of beta-adrenoceptors in human brain: A realistic goal or a mirage? *Curr Pharm Des* **10**, 1519-1536.

[19] Tanaka KF, Kashima H, Suzuki H, Ono K, Sawada M (2002) Existence of functional beta1- and beta2-adrenergic receptors on microglia. *J Neurosci Res* **70**, 232-237.

[20] Lefkowitz RJ (2007) Seven transmembrane receptors: Something old, something new. *Acta Physiol (Oxf)* **190**, 9-19.

[21] Rengo G, Zincarelli C, Femminella G, Liccardo D, Pagano G, de Lucia C, Altobelli G, Cimini V, Ruggiero D, Perrone-Filardi P, Gao E, Ferrara N, Lymperopoulos A, Koch W, Leosco D (2012) Myocardial β(2)-adrenoceptor gene delivery promotes coordinated cardiac adaptive remodelling and angiogenesis in heart failure. *Br J Pharmacol* **166**, 2348-2361.

[22] Leineweber K, Rohe P, Beilfuss A, Wolf C, Sporkmann H, Bruck H, Jakob HG, Heusch G, Philipp T, Brodde OE (2005) G-protein-coupled receptor kinase activity in human heart failure: Effects of beta-adrenoceptor blockade. *Cardiovasc Res* **66**, 512-519.

[23] Rengo G, Lymperopoulos A, Leosco D, Koch WJ (2011) GRK2 as a novel gene therapy target in heart failure. *J Mol Cell Cardiol* **50**, 785-792.

[24] Rengo G, Perrone-Filardi P, Femminella GD, Liccardo D, Zincarelli C, de Lucia C, Pagano G, Marsico F, Lymperopoulos A, Leosco D (2012) Targeting the β-adrenergic receptor system through G-protein-coupled receptor kinase 2: A new paradigm for therapy and prognostic evaluation in heart failure: From bench to bedside. *Circ Heart Fail* **5**, 385-391.

[25] Kalaria RN, Andorn AC, Tabaton M, Whitehouse PJ, Harik SI, Unnerstall JR (1989) Adrenergic receptors in aging and Alzheimer's disease: Increased beta 2-receptors in prefrontal cortex and hippocampus. *J Neurochem* **53**, 1772-1781.

[26] Lee RK, Araki W, Wurtman RJ (1997) Stimulation of amyloid precursor protein synthesis by adrenergic receptors coupled to cAMP formation. *Proc Natl Acad Sci U S A* **94**, 5422-5426.

[27] Igbavboa U, Johnson-Anuna LN, Rossello X, Butterick TA, Sun GY, Wood WG (2006) Amyloid beta-protein1-42 increases cAMP and apolipoprotein E levels which are inhibited by beta1 and beta2-adrenergic receptor antagonists in mouse primary astrocytes. *Neuroscience* **142**, 655-660.

[28] Ni Y, Zhao X, Bao G, Zou L, Teng L, Wang Z, Song M, Xiong J, Bai Y, Pei G (2006) Activation of beta2-adrenergic receptor stimulates gamma-secretase activity and accelerates amyloid plaque formation. *Nat Med* **12**, 1390-1396.

[29] Yu NN, Wang XX, Yu JT, Wang ND, Lu RC, Miao D, Tian Y, Tan L (2010) Blocking beta2-adrenergic receptor attenuates acute stress-induced amyloid beta peptides production. *Brain Res* **1317**, 305-310.

[30] Wang D, Yuen EY, Zhou Y, Yan Z, Xiang YK (2011) Amyloid beta peptide-(1-42) induces internalization and degradation of beta2 adrenergic receptors in prefrontal cortical neurons. *J Biol Chem* **286**, 31852-31863.

[31] Yu JT, Tan L, Ou JR, Zhu JX, Liu K, Song JH, Sun YP (2008) Polymorphisms at the beta2-adrenergic receptor gene influence Alzheimer's disease susceptibility. *Brain Res* **1210**, 216-222.

[32] Wang J, Ono K, Dickstein DL, Arrieta-Cruz I, Zhao W, Qian X, Lamparello A, Subnani R, Ferruzzi M, Pavlides C, Ho L, Hof PR, Teplow DB, Pasinetti GM (2011) Carvedilol as a potential novel agent for the treatment of Alzheimer's disease. *Neurobiol Aging* **32**, 1-12.

[33] Arrieta-Cruz I, Wang J, Pavlides C, Pasinetti GM (2010) Carvedilol reestablishes long-term potentiation in a mouse model of Alzheimer's disease. *J Alzheimers Dis* **21**, 649-654.

[34] Rosenberg PB, Mielke MM, Tschanz J, Cook L, Corcoran C, Hayden KM, Norton M, Rabins PV, Green RC, Welsh-Bohmer KA, Breitner JC, Munger R, Lyketsos CG (2008) Effects of cardiovascular medications on rate of functional decline in Alzheimer disease. *Am J Geriatr Psychiatry* **16**, 883-892.

[35] Fowler CJ, Garlind A, O'Neill C, Cowburn RF (1996) Receptor-effector coupling dysfunctions in Alzheimer's disease. *Ann N Y Acad Sci* **786**, 294-304.

[36] Suo WZ, Li L (2010) Dysfunction of G protein-coupled receptor kinases in Alzheimer's disease. *ScientificWorldJournal* **10**, 1667-1678.

[37] Rengo G, Lymperopoulos A, Zincarelli C, Donniacuo M, Soltys S, Rabinowitz JE, Koch WJ (2009) Myocardial adeno-associated virus serotype 6-betaARKct gene therapy improves cardiac function and normalizes the neurohormonal axis in chronic heart failure. *Circulation* **119**, 89-98.

[38] Raake PW, Vinge LE, Gao E, Boucher M, Rengo G, Chen X, DeGeorge BR Jr, Matkovich S, Houser SR, Most P, Eckhart AD, Dorn GW 2nd, Koch WJ (2008) G protein-coupled receptor kinase 2 ablation in cardiac myocytes before or after myocardial infarction prevents heart failure. *Circ Res* **103**, 413-422.

[39] Lymperopoulos A, Rengo G, Funakoshi H, Eckhart AD, Koch WJ (2007) Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure. *Nat Med* **13**, 315-323.

[40] Rengo G, Lymperopoulos A, Zincarelli C, Femminella G, Liccardo D, Pagano G, de Lucia C, Cannavo A, Gargiulo P, Ferrara N, Perrone Filardi P, Koch W, Leosco D (2012) Blockade of β-adrenoceptors restores the GRK2-mediated adrenal α(2) -adrenoceptor-catecholamine production axis in heart failure. *Br J Pharmacol* **166**, 2430-2440.

[41] Rengo G, Leosco D, Zincarelli C, Marchese M, Corbi G, Liccardo D, Filippelli A, Ferrara N, Lisanti MP, Koch WJ, Lymperopoulos A (2010) Adrenal GRK2 lowering is an underlying mechanism for the beneficial sympathetic effects of exercise training in heart failure. *Am J Physiol Heart Circ Physiol* **298**, 2032-2038.

[42] Lymperopoulos A, Rengo G, Gao E, Ebert SN, Dorn GW 2nd, Koch WJ (2010) Reduction of sympathetic activity via adrenal-targeted GRK2 gene deletion attenuates heart failure progression and improves cardiac function after myocardial infarction. *J Biol Chem* **285**, 16378-16386.

[43] Erdtmann-Vourliotis M, Mayer P, Ammon S, Riechert U, Höllt V (2001) Distribution of G-protein-coupled receptor kinase (GRK) isoforms 2, 3, 5 and 6 mRNA in the rat brain. *Brain Res Mol Brain Res* **95**, 129-137.

[44] Suo Z, Wu M, Citron BA, Wong GT, Festoff BW (2004) Abnormality of G-protein-coupled receptor kinases at prodromal and early stages of Alzheimer's disease: An association with early beta-amyloid accumulation. *J Neurosci* **24**, 3444-3452.

[45] Takahashi M, Uchikado H, Caprotti D, Weidenheim KM, Dickson DW, Ksiezak-Reding H, Pasinetti GM (2006) Identification of G-protein coupled receptor kinase 2 in paired helical filaments and neurofibrillary tangles. *J Neuropathol Exp Neurol* **65**, 1157-1169.

[46] Iaccarino G, Barbato E, Cipolletta E, De Amicis V, Margulies KB, Leosco D, Trimarco B, Koch WJ (2005) Elevated myocardial and lymphocyte GRK2 expression and activity in human heart failure. *Eur Heart J* **26**, 1752-1758.

[47] Leosco D, Fortunato F, Rengo G, Iaccarino G, Sanzari E, Golino L, Zincarelli C, Canonico V, Marchese M, Koch WJ, Rengo F (2007) Lymphocyte G-protein-coupled receptor kinase-2 is upregulated in patients with Alzheimer's disease. *Neurosci Lett* **415**, 279-282.

[48] Iadecola C (2010) The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. *Acta Neuropathol* **120**, 287-296.

[49] Obrenovich ME, Smith MA, Siedlak SL, Chen SG, de la Torre JC, Perry G, Aliev G (2006) Overexpression of GRK2 in Alzheimer disease and in a chronic hypoperfusion rat model is an early marker of brain mitochondrial lesions. *Neurotox Res* **10**, 43-56.

[50] van Marum RJ (2008) Current and future therapy in Alzheimer's disease. *Fundam Clin Pharmacol* **22**, 265-274.

[51] Thal DM, Homan KT, Chen J, Wu EK, Hinkle PM, Huang ZM, Chuprun JK, Song J, Gao E, Cheung JY, Sklar LA, Koch WJ, Tesmer JJ (2012) Paroxetine is a direct inhibitor of G protein-coupled receptor kinase 2 and increases myocardial contractility. *ACS Chem Biol* **7**, 1830-1839.