Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1768 (2007) 964–975
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbamem

Review

G protein-coupled receptor systems and their lipid environment in health disorders during aging

Regina Alemany [a,*], Javier S. Perona [b], José M. Sánchez-Dominguez [b], Emilio Montero [c], Julio Cañizares [d], Ricardo Bressani [e], Pablo V. Escribá [a], Valentina Ruiz-Gutierrez [b]

[a] *Laboratory of Molecular and Cellular Biomedicine (Associated Unit of the 'Instituto de la Grasa', CSIC), Institut Universitary d'Investigació en Ciències de la Salut (IUNICS), Department of Biology, University of the Balearic Islands, Ctra. Valldemossa Km 7.5, E-07122 Palma de Mallorca, Spain*
[b] *Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), Av. Padre García Tejero, 4, E-41012, Sevilla, Spain*
[c] *Virgen del Rocío Hospital, Av. Manuel Siurot, s/n, E-41013, Sevilla, Spain*
[d] *Residencia Heliópolis, C/ Padre Mediavilla, 2, E-41012, Sevilla, Spain*
[e] *Centro de Ciencia y Tecnología de Alimentos, Instituto de Investigación, University of Guatemala, Guatemala*

Received 19 July 2006; received in revised form 22 September 2006; accepted 27 September 2006
Available online 3 October 2006

Abstract

Cells, tissues and organs undergo phenotypic changes and deteriorate as they age. Cell growth arrest and hyporesponsiveness to extrinsic stimuli are all hallmarks of senescent cells. Most such external stimuli received by a cell are processed by two different cell membrane systems: receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs). GPCRs form the largest gene family in the human genome and they are involved in most relevant physiological functions. Given the changes observed in the expression and activity of GPCRs during aging, it is possible that these receptors are directly involved in aging and certain age-related pathologies. On the other hand, both GPCRs and G proteins are associated with the plasma membrane and since lipid–protein interactions regulate their activity, they can both be considered to be sensitive to the lipid environment. Changes in membrane lipid composition and structure have been described in aged cells and furthermore, these membrane changes have been associated with alterations in GPCR mediated signaling in some of the main health disorders in elderly subjects. Although senescence could be considered a physiologic process, not all aging humans develop the same health disorders. Here, we review the involvement of GPCRs and their lipid environment in the development of the major human pathologies associated with aging such as cancer, neurodegenerative disorders and cardiovascular pathologies.

Keywords: G protein-coupled receptor; Aging; Human pathology; Signaling protein; Lipid membrane composition; Fatty acid

Contents

* Corresponding author. Tel.: +34 971 172053; fax: +34 971 173184.
E-mail address: regina.alemany@uib.es (R. Alemany).

0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.09.024

Organisms, cells and even proteins are subject to time-dependent degenerative processes. As such, aging is characterized by the accumulation of adverse changes in cells over time, which augments the risk of disease, and the breakdown of homeostatic control and death [1]. Moreover, the process of aging is also manifested as senescence, involving the deterioration of certain functions at the cell and tissue level as well as that of the whole organism. Since aging plays an important role in many biological processes including development, tumorigenesis and death, many attempts have been made to understand the fundamental features underlying aging [2].

Cell growth arrest and hyporesponsiveness to extrinsic stimuli are hallmarks of senescent cells [3,4]. The influences of the external stimuli involved are mainly mediated by two different systems at the level of the cell membrane. One of these involves receptor tyrosine kinases (RTKs) that bind growth factors and activate signal cascades through the phosphorylation of tyrosine residues in the receptor. The other depends on G protein-coupled receptors (GPCRs) that make up approximately 5% of the genes in eukaryotic organisms [5,6]. GPCRs constitute the main family of receptors for neurotransmitters, hormones and neuromodulators. Upon agonist binding, they undergo conformational changes that result in the activation of heterotrimeric G proteins and that modulate the activity of effector proteins (e.g. adenylyl cyclase [AC], phospholipase C [PLC], A_2 [PLA_2], guanylyl cyclase [GC] and some ion channels). These effectors regulate the cytosolic levels of second messengers, which in turn influence the activity of third messengers and so on. Finally, a single signaling event may originate short-, medium-, and long-term responses that regulate a cell's activity and its responses to environmental conditions through molecular events such as the regulation of gene expression, cross-talk and other complex phenomena. After remaining active for a time, GPCRs are phosphorylated by G-protein receptor kinases (GRK) and other kinases, extinguishing the receptor's activity [7,8].

Age-dependent changes can occur at several different levels, from the ligand–receptor interaction at the cell surface to the various downstream signaling cascades with which these receptors interact. GPCRs form the largest gene family in the human genome and they are involved in most relevant physiological functions (Table 1). This fact and the changes observed in the expression and activity of GPCRs during aging indicate that these receptors may be involved in aging and associated pathologies [4].

Membrane lipids have recently been shown to influence GPCR-associated signaling [9,10] and aging [11,12]. Some specific properties of cell membranes (i.e., fluidity, nonlamellar phase propensity, surface packing, thickness, surface charge, etc.) are critical for many membrane events, such as receptor and G protein localization, activity and sorting of G protein subunits upon activation, etc. Moreover, the structure and lipid composition of the membrane regulates the function and localization of several membrane signaling proteins [10,13–17]. Changes in membrane lipid composition are frequently accompanied by alterations in the fluidity, lipid structure, and functionality of the membrane [18,19]. Accordingly, modifications in the lipid composition and the physical properties of cell membranes, in cell signaling and in gene expression have been identified in different tissues from elderly subjects, including the brain [18,20–23]. Interestingly, membrane fatty acid composition is an important determinant of the lifespan of different species [12]. In this context, alterations in membrane lipids can induce changes in the activity and localization of GPCRs in aged cells. Therefore, interventions that involve manipulating dietary lipids and lipid metabolism, including changes in diet or the administration of cholesterol-modifying agents and antioxidants, show great promise in slowing or possibly averting the development of some human diseases associated with aging including some types of cancer, Alzheimer's disease (AD), hypertension and other cardiovascular disorders [24–32].

Table 1
Some human G protein-coupled receptors (GPCRs) involved in aging and certain age-related pathologies

GPCRs	Typical localization	Response to activation
α_2-Adrenoceptors (α_{2A}, α_{2B}, α_{2C})	Noradrenergic and cholinergic nerve terminals	Inhibition of neurotransmitter (Ach, NA) release
	Platelets	Aggregation
	Smooth muscle (vascular)	Muscular contraction
	Adipocytes	Inhibition of lipolysis
β-Adrenoceptors (β_1, β_2, β_3)	Noradrenergic nerve terminals	Stimulation of NA release (β_2)
	Cardiac muscle	Positive chronotropic and ionotropic effect (β_1)
	Smooth muscle (vascular, bronchiolar)	Muscular relaxation (β_2)
	Adipocytes	Stimulation of lipolysis (β_3)
Muscarinic ACh receptors (M1, M2, M3, M4, M5)	Central nervous system, some presynaptic sites	Implication in memory and learning Stimulation (M1) or inhibition of ACh release (M2, M3)
	Cardiac muscle	Negative inotropic and chronotropic effect (M2)
	Exocrine cells	Increase exocrine secretions (M3)
Neuropeptide receptors, gastrin-releasing peptide	Central and enteric nervous system	Satiety, thermoregulation, stimulation of cell growth by autocrine feed-back (small cell lung cancer)
Somatostatin	Brain (hypothalamus)	Modulation of neurotransmission, motor and cognitive functions, reduction of amyloid β-levels
	Peripheral tissues (pituitary, pancreas, gut, thyroid, adrenal, kidney)	Inhibition of endocrine and exocrine secretions
Thyrotropin receptors	Thyroid	Stimulation of thyroid hormone release, increase of number, size and activity of follicular cells

Although senescence could be considered a physiological process, not all aging individuals develop the same health disorders. Here we shall review the involvement of GPCRs in the development of major human pathologies in aging, such as cancer, cardiovascular and neurodegenerative disorders (mainly AD). We shall focus on the alterations of membrane lipid composition and structure associated with these pathologies, its influence on GPCR signaling, and the beneficial effects of some fatty acid-rich diets on healthy aging.

1. GPCRs and aging in cancers

In general, there is a positive correlation between age and the incidence of cancer, in particular in breast, lung, prostate, and colon cancers [33,34]. Cancers originate through defects in the control of cell proliferation, usually associated with mutations in proto-oncogenes, tumor suppressors and other signaling proteins. Obviously, the probability that a cell might bear a mutation increases with age and indeed, many cancers require the accumulation of multiple mutations. For instance, p53 induces apoptosis of cells with genetic alterations that cannot be repaired and mutations in p53 have been observed in about 50% of all human tumors. However, p53 alone does not induce uncontrolled cell proliferation, but rather the deficiency in p53 function allows a cell with mutation in genes involved in proliferation to divide continuously. It is therefore clear that in most cancers, the probability of developing a tumor increases with age (Fig. 1) [35]. Nevertheless, it should be noted that exceptions to this rule do exist, as is the case for pilocytic astrocytoma (Fig. 1).

With respect to GPCRs and their related proteins, the role they fulfill in the control of cell proliferation is closely associated with their involvement in the development of certain cancers [36–38]. Indeed, changes in the expression and function of these receptors may result in cellular transformation [39]. Recent studies have demonstrated that GPCRs are implicated in tumorigenesis and metastasis [40]. Changes in the expression of GPCRs or mutations in the genes encoding these receptors have been observed in some cancers, indicating that certain GPCRs can behave as potent agonist-dependent oncogenes [41,42].

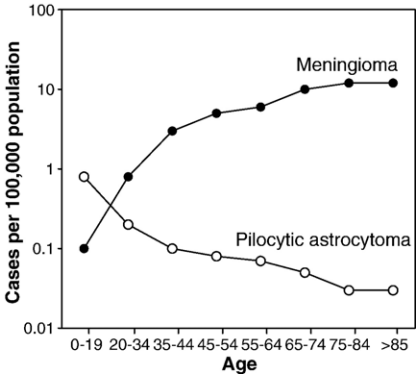

Fig. 1. Age-related incidence of cancer. The figure shows an example of the increase in the rate of a specific cancer with age (meningioma), representing the usual trend for most cancers, and an example of a cancer whose prevalence decreases with age (pilocytic astrocytoma). Adapted from Wrensch et al. 2002.

One example of a growth-stimulatory role of GPCRs can be seen in small cell lung carcinoma (SCLC) cells, where neuropeptides like gastrin-releasing peptide (GRP), galanin, vasopressin, etc., activate GPCRs [43–45]. SCLC constitutes approximately 25% of all lung cancers and it is characterized by a very low 5-year survival rate, despite the initial sensitivity that it displays to radio- and chemotherapy. In SCLC, neuropeptides activate GPCRs that are coupled to more than one G protein family (i.e., Gq/11 and G12/13) [41] thereby stimulating phospholipase C [46] and ERK [47] activity, which promotes cell proliferation. Thus, through an autocrine feedback on GPCRs neuropeptides represent the principal mitogens of SCLC [44] and indeed, neuropeptide growth factor antagonists have been developed to treat SCLC [48].

The detection of activating mutations of the human thyrotropin (TSH) receptor in some types of thyroid carcinomas established a causal link between GPCRs and autonomous cell growth [49]. The human TSH receptor has an exceptionally broad profile of G protein coupling and it is able to interact with members of all four G protein families, Gs, Gi, Gq and G12 [50,51]. The cAMP regulatory cascade has also been implicated in the control of growth and differentiation, whereas calcium and diacylglycerol (G_q/phospholipase C-β activation) are thought to stimulate iodination and thyroid hormone synthesis [52]. In approximately 30% of hyperfuctional thyroid adenomas, GTPase-inhibiting mutations in Gαs are responsible for the increased cAMP synthesis characteristic of this tumor. Similarly, mutations in the THS receptor constitutively activate its ability to stimulate G- and AC-catalyzed cAMP synthesis [53–55]. Moreover, alterations in the desensitization of GPCRs in the thyroid gland play a crucial role in thyroid pathologies [56]. In differentiated thyroid carcinomas (where TSH acts as a mitogenic agent), changes in the levels of GRK5 are correlated with a decrease in the desensitization of the TSH receptor that provokes an increase in cAMP synthesis [57]. Likewise, an increase in GRK3 expression was detected in hyperfunctional thyroid nodules (HTNs), suggesting a potential role for this GRK as a negative feedback regulator for the constitutively activated cAMP pathway in these structures [56]. Similar alterations in GPCR and GRK activation and/or expression are associated with the development of breast cancer [58–61], with neoplastic transformation in the prostate [62–65] and in human colon cancer [66]. All these cancers become more prevalent with age, most probably due to their dependence on the accumulation of mutations.

Taken together, these results show that mutations in GPCRs, G proteins, their effectors and GRKs are all involved in the etiology of several human cancers, particularly those involving hormone dependence. Hence, it would appear that these proteins might be relevant pharmacological targets for selective strategies aimed at antagonizing distinct mitogenic stimuli. Tumor growth and metastasis are both processes that are affected by changes in membrane lipid composition and accordingly, lipid alterations might be involved in the development of some types of cancer. In this context, a wide variety of molecular entities that control cell proliferation and survival are membrane-associated proteins. Indeed, alterations in the

levels of certain membrane lipids have been observed in cell membranes from patients with cancer and from cancer cells that are resistant to chemotherapy [13,67,68]. Interestingly, compounds that modulate the membrane lipid structure (e.g. fatty acids) influence the cellular localization and activity of important membrane-associated signal transduction proteins [10,14,15,69], thereby producing molecular and cellular alterations that affect cell signaling and division [70,71]. Thus, it is not surprising that the activity of various anticancer drugs is associated with their ability to alter membrane lipid composition, thereby affecting membrane lipid structure. Accordingly, the possibility of using lipids as targets to overcome anticancer drug resistance has recently been highlighted [67,72].

2. GPCRs and aging in the brain under normal conditions and in association with neurodegenerative disorders

A variety of age-related alterations in GPCRs have been observed in neuronal signaling systems [73]. While age-associated changes appear to be variable and dependent on the specific brain region, the expression of most GPCRs and G proteins decreases in the human brain with age [74–77]. Indeed, like most brain neurotransmitter receptors, the density of β-adrenoceptors decreases with age in the human brain [78]. This decline is largely associated with the degeneration of noradrenergic nerve terminals [79] and it is mainly due to the loss of high-affinity receptors [76]. The human brain contains all three subtypes of α_2-adrenoceptors identified by molecular cloning (α_{2A}, α_{2B}, and α_{2C}), the α_{2A}-adrenoceptor being the most abundant in the frontal cortex [75]. The density of α_{2A}-adrenoceptors and associated regulatory G proteins has also been shown to decrease with aging in the human brain [74,75,80]. The concomitant decrease in the density of α_{2A}- and β-adrenoceptors in the frontal cortex suggests a relationship between both these GPCRs [76]. Both adrenoceptors belong to a large family of membrane receptors that regulates AC activity through Gαs and Gαi proteins [81–83]. Some studies reveal a decline of various Gα proteins and their signal transduction cascades within the aging human brain [75,76,84,85]. This coordinated decrease in the density of β- and α_{2A}-adrenoceptors indicates the existence of a relationship between these receptors and their signaling proteins [76]. Furthermore, the muscarinic acetyl choline (ACh) receptor (mAChR) system loses its sensitivity to ACh stimulation both during aging and in AD [73,86,87]. In contrast to most receptors and signaling proteins, the presence of other receptors and signaling-related proteins may also increase with age, as seen for the imidazoline receptors and monoamine oxidase [88,89]. This would indicate that the loss of such elements is not unspecific.

AD is currently the commonest cause of senile dementia among humans over 65, and it is characterized by a progressive cognitive decline coupled defined by the loss of memory, cognitive abilities, and even of personality. These changes are due to the progressive dysfunction and death of neurons responsible for learning and memory. AD is characterized by a variety of pathological features, such as the extracellular senile plaques formed by amyloid β peptides, the appearance of intracellular neurofibrillary tangles consisting of twisted filaments of hyperphosphorylated tau protein, the loss of neuronal subpopulations and cholinergic fibers, and brain atrophy [90–94]. The amyloid β precursor protein (APP) is a membrane protein and the proteolytic fragments generated during AD form deposits on the extracellular face of the cell membrane. It has recently been demonstrated that a gradual decline in the cellular processing and maturation of APP is one of the main pathogenic mechanisms for familial and age-associated AD [95]. GPCRs have recently been implicated in APP processing. In this context, the somatostatin receptor has been identified as a modulator that decreases amyloid β levels by increasing brain neprilysin activity [96], a rate-limiting peptidase involved in the physiological degradation of amyloid β in the brain [97]. Thus, reduced neprilysin activity contributes to amyloid β accumulation and consequently, to AD development. There is evidence that neprilysin is down-regulated in the hippocampus and cerebral cortex with aging [98] and from an early stage of AD development [99–101], supporting the association of neprilysin activity with the etiology and pathogenesis of AD. Therefore, the up-regulation of neprilysin through the somatostatin-activated GPCR signaling pathway may represent a promising target for AD therapeutic and preventative strategies [102].

Postsynaptic M1 AChRs are found at high density in the neocortex and hippocampus, where they mediate cholinergic neurotransmission in a variety of CNS functions including learning and memory [103]. Of the neurotransmitter pathologies associated with AD, cholinergic dysfunction occurs early in the disease process [104] and it is thought to underlie much of the characteristic cognitive and neuropsychiatric symptoms [105–107]. In addition, the ability of the mAChR to form high affinity agonist-binding complexes with G proteins was impaired in AD [108] and phosphoinositide hydrolysis, as well as phospholipase C and PKC activity was reduced [109,110]. Recently, the reduction in M1 AChR/G-protein coupling has been related to the severity of cognitive symptoms in the neocortex of AD patients. Thus, that impairment of M1 AChR-mediated signaling through uncoupling of its G protein may be a neurochemical cause of cognitive decline in AD [111]. Potential mechanisms for the loss of GPCR-G protein coupling in AD may be aberrant phosphorylation or dephosphorylation of these receptors, which in turn may alter their association with G proteins and/or the levels or activity of G proteins (e.g., loss of GTPase activity). Accordingly, both G protein subunit concentrations and activity are altered in different regions of the AD brain, thereby modifying GPCR-mediated AC signaling. In fact, it has been reported that inhibition of AC mediated by Gs protein but not Gi is disrupted in various brain regions in AD patients [112–116]. Furthermore, the interactions between the β_1-adrenoceptor and Gs protein are disrupted in the temporal cortex in AD [117] and β-adrenoceptor-stimulated cAMP production is reduced in fibroblasts from sporadic AD cases [118]. Finally, a deficit in cytosolic PKA has been associated with the accumulation of neurofibrillary changes and amyloid β deposits in the AD brain [119].

The fatty acid composition of neural tissue plays an important role in neurodevelopment, changing significantly during periods of rapid brain growth [120]. Indeed, neural tissue has the highest lipid content after adipose tissue. The levels of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) in the cerebral cortex change from early childhood through late adulthood [121], and some studies suggest that neurocognitive function may be related to the fatty acid composition of developing brain [122–124]. Accordingly, alterations in brain fatty acid composition have been reported in several diseases associated with neurodegenerative disorders [125]. Neurodegeneration in AD is accompanied by lipid alterations, such as changes in the phosphocholine-containing lipids in cerebrospinal fluid [126], and in the levels of phospholipids and fatty acid in blood plasma and brain membranes [127,128]. Furthermore, several aspects related to the etiology of AD are associated with alterations of lipid membrane composition and structure. On the one hand, levels of plasmalogens (the major ethanolamine glycerophospholipids in the brain) and long-chain ω-3 PUFAs (e.g., docohexaenoic acid) decrease in brains of AD patients, which is proposed to provoke membrane bilayer destabilization contributing in neurodegeneration [129]. This effect may act cooperatively with the amyloid cascade mechanism, by rendering APP. Accordingly, recent results demonstrate that age-dependent membrane modifications are associated with alteration in the distribution of presenilin-1 and a beta-APP-cleaving enzyme, very likely affecting APP processing and leading to its accumulation [95]. On the other hand, some membrane alterations could in part explain the cognitive deficits reported in mice with neurodegenerative disorders [130,131], as well as the impaired neurotransmitter-associated signaling observed in AD and aging [132,133]. These specific alterations in membrane lipids have led to studies on the effects of phospholipid and fatty acid supplementation on the mental status of patients with AD and in animal models of aging [28,29,134,135]. Diets rich in carbohydrate [136], particularly those with a high glycemic index, and those low in essential fatty acids (particularly ω-3 long-chain PUFAs) increase the risk of developing AD [137–140]. Likewise, the risk of AD may be increased through altered lipid metabolism and neuronal membrane lipid composition that may lead to changes in neurotransmission, antioxidant defenses, inflammatory responses, cerebral blood flow, and cognitive function [20,27,31,141,142]. Of particular interest are the beneficial anti-oxidant and anti-inflammatory effects of dietary ω-3 PUFAs, which could be a promise for the prevention of AD [27]. Similarly, essential components of the Mediterranean diet protect against age-related cognitive decline [143,144], as well as protecting against atherosclerosis in an animal model of neurodegeneration [145]. Furthermore, there is evidence that this diet also benefits inflammatory and cardiovascular parameters [25].

3. GPCRs and aging in cardiovascular disorders

Adrenergic receptors are important regulators of cardiovascular physiology. The β-adrenergic receptor is activated by both noradrenaline and adrenaline, and it is a target for many medications prescribed to the elderly to treat hypertension, angina, post-myocardial infarction risk, congestive heart failure, glaucoma, tremor, and arrhythmias [146]. The β-adrenergic receptor also exhibits age-related change in its activity in the cardiovascular system.

3.1. In the heart

Human aging is associated with an increase in the activity of the sympathetic nervous system and plasma catecholamine levels [147–150]. In this situation, the cardiac adrenoceptors are exposed to chronic stimulation and hence, desensitization of cardiac β-adrenoceptors could be expected with age (for a review see [151]). Numerous studies in animals show that the cardiac responses mediated by the β-adrenoceptor diminish with age. Although such changes in the density of the β-adrenoceptor in the myocardium with age are not consistent (decrease, increase and no changes have been reported), impaired coupling of the β-adrenoceptor to the Gs protein and to the catalytic unit of the adenylyl cyclase (AC) with age was consistently observed [152,153].

In humans and animals, a decrease in the levels of the β_1-adrenoceptor and Gs protein in the ventricular myocardium appear to be responsible for the reduction in β-adrenoceptor activity in aging [154]. In contrast, the increase in Gi protein levels and the reduction in the activity of the catalytic subunit of the AC lead to attenuated cyclic AMP formation in aged right atrial tissue [155]. However, irrespective of the underlying mechanism, the responses of the aged human heart to β-adrenoceptor stimulation and to stimulation by other receptors (i.e., serotonin 5-HT_4 receptors and histamine H_2 receptors) that evoke their effects through activation of AC are impaired. The density of muscarinic receptors also decreased with age in the human heart, and there was a significant inverse correlation between muscarinic receptor density and the age of the subjects. This decrease in receptor density was accompanied by an impairment in carbachol-induced inhibition of AC and an attenuation of the indirect negative inotropic effect of carbachol with aging [156,157].

Taken together, it appears that autonomic receptor systems are altered in the aging human heart to protect the heart against a pronounced reduction in β-adrenoceptor responsiveness. Indeed, β-adrenoceptors and muscarinic receptors are both desensitized in parallel, thereby leading to an unaltered in vivo response of β-adrenoceptor stimulation. Furthermore, GRKs activity does not change [158] and therefore does not contribute to (or exaggerate) β-adrenoceptor desensitization.

3.2. In the vasculature

Hypertension, orthostatic hypotension, arterial insufficiency, and atherosclerosis are common disorders in the elderly, with a significant morbidity and mortality. It is generally understood that with advancing age, vascular tone shifts towards vasoconstriction and producing a hypertensive state. This shift is related to the precipitous decline of vasorelaxation with advanced age, stimulated by activation of Gαs-linked receptors (β-adrenergic,

prostanglandin E_2, adenosine A_2, etc.). Therefore, research has been focused on age-related changes in GPCR function, and specifically on the β-adrenergic receptor cascade [159,160].

In vascular smooth muscle cells the generation of the vasodilatory agent, cAMP, by AC activation is due to agonist stimulation of GαsPCRs (β-adrenergic, prostanglandin-E_2, adenosine A_2, etc.). Activation of PKA by cAMP initiates vasorelaxation through different pathways, all of which lead to a lowering of cytosolic Ca^{2+} [161]. During aging, β-adrenoceptor-mediated function and the subsequent generation of cAMP in the vasculature declines, leading to impaired vasorelaxation. Because cAMP is also an anti-proliferative agent, this age-related decrease may be associated with the progress of atherosclerosis. The accepted explanation for the age-related lack of β-adrenoceptor function is that GRK and β-arrestin expression is upregulated, leading to a higher basal phosphorylation and desensitization of the receptors [162,163]. Numerous studies demonstrate that Gαs may also undergo age-related changes that impair its coupling to β-adrenoceptor after agonist-binding, decreasing the proportion of high affinity receptors [164,165]. However, the total β-adrenergic receptor pool [162,166] and the expression of Gαs in the vasculature remains unchanged with age [162,167–169]. Further studies of the changes in G protein function with age in vascular tissue are needed to determine whether changes in Gαs function underlie the loss of β-adrenoceptor-mediated vasorelaxation. A recent report has identified a novel regulator of G protein signaling (RGS)/GTPase activating protein (GAP) that acts on Gαs [170]. One possible explanation for the age-related alterations associated with Gαs is that RGS/GAP activity is enhanced in vessels from older animals. Under these conditions, agonist exposure would leave β-adrenoceptors in a low-affinity state due to their dissociation from Gαs. Thus, Gαs would not stimulate AC to produce cAMP, as Gαs signaling would be quenched due to the high GAP activity of the RGS/GTPase activating protein.

Alterations in the membrane content of cholesterol or phospholipid, in the phospholipid distribution, in the molecular species of particular phospholipid classes, and in the degree of fatty acid saturation have also been reported in hypertensive humans [171–173]. Interestingly, alterations in cell membrane lipid levels are associated with a reduction in the density of membrane-associated signaling proteins involved in the control of blood pressure, such as G proteins and PKC in elderly hypertensive subjects [23]. Therefore, it appears that one way to regulate GPCR signaling in the elderly would be through nutritional and pharmacologic interventions aiming at normalizing the abnormal lipid composition of the plasma membrane.

The interest in the potential cardiovascular health benefits of dietary MUFAs has increased in recent years [174–176]. It has been demonstrated that in hypertensive normocholesterolemic and hypercholesterolemic subjects, high oleic acid intake (i.e. olive oil) lowers blood pressure [176]. Moreover, high oleic acid intake also normalizes certain alterations in erythrocyte membrane function in hypertensives, such as the distribution of the erythrocyte Na^+–Li^+ countertransport [172] and membrane cholesterol [177–179]. Normalization of these parameters by olive oil is concomitant with a reduction in blood pressure and strongly related to lipoprotein and membrane lipid modifications [30,176,180,181]. In addition, oleic acid regulates the activity of adrenoceptor, G protein and adenylyl cyclase activities [71], and the GPCR pathway involved in the control of blood pressure.

In elderly hypertensive subjects, long-term olive oil consumption reduces the cholesterol/phospholipid ratio in erythrocyte membranes (before olive oil consumption 0.44±0.00 and after 0.38±0.01, $P<0.05$), normalizing these values to those of normotensives (0.37±0.01). These decreases in the levels of cholesterol in membranes after olive oil consumption are associated with increased membrane fluidity [182]. Accordingly, a reduction in membrane fluidity (high cholesterol) has been associated with the development of hypertension [183] and with an age-related impairment of β-adrenergic-mediated vasorelaxation and Gαs coupling [22,164,184]. Olive oil consumption also induces significant changes in the levels of specific fatty acid moieties in phospholipids and cholesterol esters (Table 2). In both cases, MUFA levels significantly increase in elderly humans after long-term olive oil consumption, mainly due to a rise in the proportion of oleic acid (C18:1). This fact was reflected in a significant increase of the MUFA:SFA (saturated fatty acid) ratio (from 0.57 to 0.65 in the normotensive group and from 0.52 to 0.65 in the hypertensive subjects) and of the MUFA:PUFA ratio in membrane phospholipids (from 0.62 to 0.71 in normotensive subjects and from 0.57 to 0.72 in hypertensive subjects). In contrast, the PUFA:SFA ratio did not markedly change under these circumstances. These changes induce important alterations in membrane lipid structure because oleic acid favors the formation of nonlamellar membrane structures in vitro (hexagonal H_{II} phases) [9,185]. The H_{II}-phase propensity is an

Table 2
Fatty acid composition of phospholipids in erythrocyte membranes (mg/100 mg)

Fatty acid species	Normotensives		Hypertensives	
	Basal	Long-term olive oil	Basal	Long-term olive oil
14:1	2.21±0.26	2.49±0.16	1.84±0.22	2.62±0.14†
16:0	22.93±0.45	24.08±0.86	24.00±0.67	25.76±0.49
16:1	0.81±0.08	0.80±0.07	0.85±0.08	0.93±0.08
18:0	16.88±0.82	14.56±0.63*	16.42±0.29	13.31±0.68†
18:1	17.49±0.42	20.00±1.01*	17.90±0.79	19.30±0.93
18:2	13.25±0.49	12.05±0.60	12.75±0.79	12.43±0.70
18:3	0.73±0.03	0.42±0.03†	0.68±0.06	0.52±0.05*
20:2	2.14±0.17	2.35±0.22	2.22±0.14	2.14±0.12
20:4	16.98±0.44	16.02±0.73	16.81±0.34	15.47±0.62*
22:5	3.47±0.18	3.30±0.21*	3.28±0.24	2.86±0.12
Others	3.12±0.12	3.92±0.16	2.40±0.22	3.85±0.32†
Total SFA	39.99±0.66	38.89±0.89	40.73±0.59	39.3±0.72
Total MUFA	22.91±0.54	25.36±0.42†	21.42±0.66	25.51±0.47†
Total PUFA	37.10±0.57	35.73±0.55†	37.85±0.68	35.18±0.72†
MUFA:SFA	0.57±0.07	0.65±0.06	0.52±0.05	0.65±0.06†
MUFA:PUFA	0.62±0.05	0.71±0.06*	0.57±0.04	0.72±0.06†
PUFA:SFA	0.93±0.04	0.92±0.03	0.93±0.03	0.90±0.04

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values are expressed as the mean±SEM ($n=28$). *$P<0.05$, $^{\dagger}P<0.01$, when compared to the corresponding basal group.

important physical property of cell membranes, since it influences the localization and activity of several membrane-associated proteins (e.g., G proteins and PKC) [10,14,70,71]. Both the type and the quantities of free or esterified fatty acids and cholesterol influence the membrane fluidity and H_{II}-phase propensity [9]. In this context, the normotensive effects of olive oil could originate from the modulation of H_{II}-phase propensity (induced by changes in membrane lipids) and its influence on the interaction of G proteins and PKC, in addition to or alternative to membrane fluidity. Long-term high oleic acid intake significantly reduces the membrane levels of $G\alpha i_{1/2}$, $G\alpha s$, $G\beta$ and $PKC\alpha$ in elderly hypertensive subjects (Fig. 2). These effects are accompanied by a reduction in blood pressure (mean systolic and diastolic blood pressure values were 162.4 and 81.0 mm Hg, respectively before and 138.0 and 72.3 mm Hg after olive oil consumption, $P<0.05$), suggesting that the changes observed may in part account for the normotensive effects of olive oil. Moreover, these results support the hypothesis that the lower basal levels of membrane-associated $G\alpha i_{1/2}$, $G\alpha o$ and $PKC\alpha$ previously found in elderly hypertensive subjects [23] result from the compensatory adaptation to other changes that induce hypertension, rather than to the etiology of this pathology. Oleic acid has been shown to regulate α_2-adrenoceptors and related signaling proteins in 3T3 cells, whereas the chemically related analogues had no effect [71]. This effect has been related to the structural regulation of the membrane induced by this fatty acid and it could explain the effects induced by high olive oil consumption. Moreover, oleic acid derivatives have been found to exert a normotensive action in hypertensive animals [186].

4. Conclusions

There is currently strong evidence that the function and expression of GPCRs and other related signaling proteins are affected by age. Thus, altered GPCR signaling may be involved

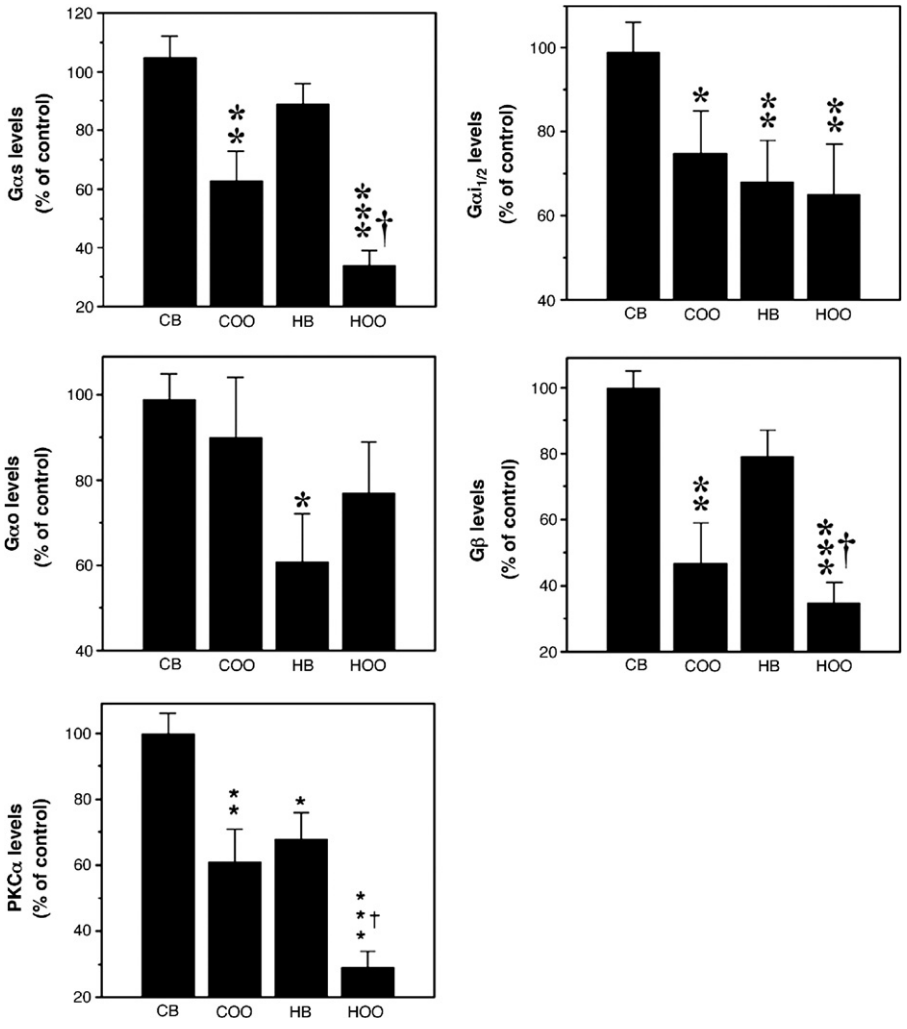

Fig. 2. G protein and PKCα concentrations in erythrocyte membranes of elderly subjects. The data shown are the mean±SEM of the G protein densities ($n=10$). CB indicates basal values in control (normotensive) subjects; COO, controls after olive oil consumption; HB, basal values in hypertensive subjects; HOO, hypertensive subjects after long-term olive oil consumption. Panels show the levels of Gαs, $G\alpha i_{1/2}$, Gαo, Gβ and PKCα. Quantification was performed by image analysis, using standard curves with four points (i.e., total protein loaded vs. integrated optical density) of different protein contents loaded on the same gels as described [23]. Representative immunoblotting bands are also shown. About 36 μg of total protein was loaded for all subjects. *$P<0.05$; **$P<0.01$; ***$P<0.001$, vs. CB; †$P<0.01$ vs. HB.

in many important age-related human diseases, either as a consequence of alterations suffered by senescent cells or due to its participation in the origin or the development of the disease. Furthermore, it has recently been hypothesized that aging could be initiated and modified at the plasma membrane, leading to the proposal that the membrane might act as a gate which modulates the signals that induce a senescent phenotype [12]. Thus, aging may alter the lipid composition and structure of biological membranes, which in turn regulate the activity of membrane proteins involved in cell signaling and important physiologic functions, such as growth, neurotransmission and blood pressure. In this context, small heat shock proteins can act as membrane lipid sensors capable of recognizing membrane lipid structures and of modulating them [187]. Moreover, the possibility of reversing the senescent phenotype by simply restoring the membrane signaling apparatus confirms the significance of membrane in the aging [4]. From these results, it might be hypothesized that the age associated decline of GPCR signaling could be initiated and modified at the membrane level and consequently, one way to restore the effects of this process in elderly humans would be to modify membrane lipid composition and structure. In this sense, fats are important in human diet and indeed, different types of food lipids have been associated with both positive and negative effects on human health. As such, dietary fatty acids, among others the long-chain ω-3 PUFAs and the MUFA oleic acid component of the Mediterranean diet (i.e., olive oil), have been shown to have beneficial effects in important human pathologies linked to age, such as AD, hypertension and related cardiovascular disorders [29,30,32].

Acknowledgments

Work in the authors' laboratories is supported by Grants FEDER 1FD97-2288 (European Community), SAF2001-0839, SAF2003-00232 and SAF 2004-05249 (Ministerio de Ciencia y Tecnología, Spain) and FIS/PI03-1218 (Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III). We are grateful to the director, nurses and medical personnel of the 'Residencia de la Tercera Edad Heliópolis' for their cooperation during this study. Regina Alemany and Javier S. Perona hold contracts from the Ramón y Cajal and Juan de la Cierva Programmes, respectively.

References

[1] D. Harman, Aging: overview, Ann. N. Y. Acad. Sci. 928 (2001) 1–21.
[2] J.R. Smith, O.M. Pereira-Smith, Replicative senescence: implications for in vivo aging and tumor suppression, Science 273 (1996) 63–67.
[3] E.J. Yeo, I.S. Jang, H.K. Lim, K.S. Ha, S.C. Park, Agonist-specific differential changes of cellular signal transduction pathways in senescent human diploid fibroblasts, Exp. Gerontol. 37 (2002) 871–883.
[4] E.J. Yeo, S.C. Park, Age-dependent agonist-specific dysregulation of membrane-mediated signal transduction: emergence of the gate theory of aging, Mech. Ageing Dev. 123 (2002) 1563–1578.
[5] C.I. Bargmann, Neurobiology of the *Caenorhabditis elegans* genome, Science 282 (1998) 2028–2033.
[6] A. Marchese, S.R. George, L.F. Kolakowski Jr., K.R. Lynch, B.F. O'Dowd, Novel GPCRs and their endogenous ligands: expanding the boundaries of physiology and pharmacology, Trends Pharmacol. Sci. 20 (1999) 370–375.
[7] W.P. Hausdorff, M.G. Caron, R.J. Lefkowitz, Turning off the signal: desensitization of beta-adrenergic receptor function, FASEB J. 4 (1990) 2881–2889.
[8] J.G. Krupnick, J.L. Benovic, The role of receptor kinases and arrestins in G protein-coupled receptor regulation, Annu. Rev. Pharmacol. Toxicol. 38 (1998) 289–319.
[9] S.S. Funari, F. Barcelo, P.V. Escriba, Effects of oleic acid and its congeners, elaidic and stearic acids, on the structural properties of phosphatidylethanolamine membranes, J. Lipid Res. 44 (2003) 567–575.
[10] O. Vogler, J. Casas, D. Capo, T. Nagy, G. Borchert, G. Martorell, P.V. Escriba, The Gbetagamma dimer drives the interaction of heterotrimeric Gi proteins with nonlamellar membrane structures, J. Biol. Chem. 279 (2004) 36540–36545.
[11] M. Choe, C. Jackson, B.P. Yu, Lipid peroxidation contributes to age-related membrane rigidity, Free Radic. Biol. Med. 18 (1995) 977–984.
[12] A.J. Hulbert, On the importance of fatty acid composition of membranes for aging, J. Theor. Biol. 234 (2005) 277–288.
[13] P.V. Escriba, A.V. Ferrer-Montiel, J.A. Ferragut, J.M. Gonzalez-Ros, Role of membrane lipids in the interaction of daunomycin with plasma membranes from tumor cells: implications in drug-resistance phenomena, Biochemistry 29 (1990) 7275–7282.
[14] P.V. Escriba, M. Sastre, J.A. Garcia-Sevilla, Disruption of cellular signaling pathways by daunomycin through destabilization of nonlamellar membrane structures, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 7595–7599.
[15] J. Giorgione, R. Epand, C. Buda, T. Farkas, Role of phospholipids containing docosahexaenoyl chains in modulating the activity of protein kinase C, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 9767–9770.
[16] G. Basanez, J.L. Nieva, F.M. Goni, A. Alonso, Origin of the lag period in the phospholipase C cleavage of phospholipids in membranes. Concomitant vesicle aggregation and enzyme activation, Biochemistry 35 (1996) 15183–15187.
[17] H. Ahyayauch, A.V. Villar, A. Alonso, F.M. Goni, Modulation of PI-specific phospholipase C by membrane curvature and molecular order, Biochemistry 44 (2005) 11592–11600.
[18] J. Zicha, J. Kunes, M.A. Devycnk, Abnormalities of membrane function and lipid metabolism in hypertension, Am. J. Hypertens. 12 (1999) 315–331.
[19] R. Buchet, S. Pikula, Alzheimer's disease: its origin at the membrane, evidence and questions, Acta Biochim. Pol. 47 (2000) 725–733.
[20] K. Wells, A.A. Farooqui, L. Liss, L.A. Horrocks, Neural membrane phospholipids in Alzheimer disease, Neurochem. Res. 20 (1995) 1329–1333.
[21] P. Caprari, A. Scuteri, A.M. Salvati, C. Bauco, A. Cantafora, R. Masella, D. Modesti, A. Tarzia, V. Marigliano, Aging and red blood cell membrane: a study of centenarians, Exp. Gerontol. 34 (1999) 47–57.
[22] J.M. Noble, T.H. Thomas, G.A. Ford, Effect of age on plasma membrane asymmetry and membrane fluidity in human leukocytes and platelets, J. Gerontol., A, Biol. Sci. Med. Sci. 54 (1999) M601–M606.
[23] P.V. Escriba, J.M. Sanchez-Dominguez, R. Alemany, J.S. Perona, V. Ruiz-Gutierrez, Alteration of lipids, G proteins, and PKC in cell membranes of elderly hypertensives, Hypertension 41 (2003) 176–182.
[24] J.M. Martin-Moreno, W.C. Willett, L. Gorgojo, J.R. Banegas, F. Rodriguez-Artalejo, J.C. Fernandez-Rodriguez, P. Maisonneuve, P. Boyle, Dietary fat, olive oil intake and breast cancer risk, Int. J. Cancer 58 (1994) 774–780.
[25] R. De la Puerta, E. Martinez-Dominguez, V. Ruiz-Gutierrez, Effect of minor components of virgin olive oil on topical antiinflammatory assays, Z. Naturforsch., C 55 (2000) 814–819.
[26] L.A. Ferrara, A.S. Raimondi, L. d'Episcopo, L. Guida, A. Dello Russo, T. Marotta, Olive oil and reduced need for antihypertensive medications, Arch. Intern. Med. 160 (2000) 837–842.
[27] G.P. Lim, T. Chu, F. Yang, W. Beech, S.A. Frautschy, G.M. Cole, The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse, J. Neurosci. 21 (2001) 8370–8377.
[28] G. Barcelo-Coblijn, E. Hogyes, K. Kitajka, L.G. Puskas, A. Zvara, L.

Hackler Jr., C. Nyakas, Z. Penke, T. Farkas, Modification by docosahexaenoic acid of age-induced alterations in gene expression and molecular composition of rat brain phospholipids, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 11321–11326.
[29] G. Barcelo-Coblijn, K. Kitajka, L.G. Puskas, E. Hogyes, A. Zvara, L. Hackler Jr., T. Farkas, Gene expression and molecular composition of phospholipids in rat brain in relation to dietary n-6 to n-3 fatty acid ratio, Biochim. Biophys. Acta 1632 (2003) 72–79.
[30] J.S. Perona, J. Canizares, E. Montero, J.M. Sanchez-Dominguez, A. Catala, V. Ruiz-Gutierrez, Virgin olive oil reduces blood pressure in hypertensive elderly subjects, Clin. Nutr. 23 (2004) 1113–1121.
[31] R.M. Lane, M.R. Farlow, Lipid homeostasis and apolipoprotein E in the development and progression of Alzheimer's disease, J. Lipid Res. 46 (2005) 949–968.
[32] R. Estruch, M.A. Martinez-González, D. Corella, J. Salas-Salvadó, V. Ruiz-Gutierrez, M.I. Covas, M. Fiol, E. Gómez-Gracia, M.C. López-Sabater, E. Vinyoles, F. Arós, M. Conde, C. Lahoz, J. Lapetra, G. Sáez, E. Ros, Effects of a Mediterranean-style diet on cardiovascular risk factors, Ann. Int. Med. 145 (2006) 1–11.
[33] I. Carreca, L. Balducci, M. Extermann, Cancer in the older person, Cancer Treat. Rev. 31 (2005) 380–402.
[34] L.C. Walter, C.L. Lewis, M.B. Barton, Screening for colorectal, breast, and cervical cancer in the elderly: a review of the evidence, Am. J. Med. 118 (2005) 1078–1086.
[35] M. Wrensch, Y. Minn, T. Chew, M. Bondy, M.S. Berger, Epidemiology of primary brain tumors: current concepts and review of the literature, Neuro-oncol. 4 (2002) 278–299.
[36] T. van Biesen, L.M. Luttrell, B.E. Hawes, R.J. Lefkowitz, Mitogenic signaling via G protein-coupled receptors, Endocr. Rev. 17 (1996) 698–714.
[37] N. Dhanasekaran, M.V. Prasad, G protein subunits and cell proliferation, Biol. Signals Recept. 7 (1998) 109–117.
[38] N. Dhanasekaran, S.T. Tsim, J.M. Dermott, D. Onesime, Regulation of cell proliferation by G proteins, Oncogene 17 (1998) 1383–1394.
[39] J.S. Gutkind, Cell growth control by G protein-coupled receptors: from signal transduction to signal integration, Oncogene 17 (1998) 1331–1342.
[40] S. Li, S. Huang, S.B. Peng, Overexpression of G protein-coupled receptors in cancer cells: involvement in tumor progression, Int. J. Oncol. 27 (2005) 1329–1339.
[41] N. Dhanasekaran, L.E. Heasley, G.L. Johnson, G protein-coupled receptor systems involved in cell growth and oncogenesis, Endocr. Rev. 16 (1995) 259–270.
[42] T. Gudermann, R. Grosse, G. Schultz, Contribution of receptor/G protein signaling to cell growth and transformation, Naunyn-Schmiedeberg's Arch. Pharmacol. 361 (2000) 345–362.
[43] T. Sethi, S. Langdon, J. Smyth, E. Rozengurt, Growth of small cell lung cancer cells: stimulation by multiple neuropeptides and inhibition by broad spectrum antagonists in vitro and in vivo, Cancer Res. 52 (1992) 2737s–2742s.
[44] E. Rozengurt, Autocrine loops, signal transduction, and cell cycle abnormalities in the molecular biology of lung cancer, Curr. Opin. Oncol. 11 (1999) 116–122.
[45] J.C. Reubi, Peptide receptors as molecular targets for cancer diagnosis and therapy, Endocr. Rev. 24 (2003) 389–427.
[46] A. Beekman, B. Helfrich, P.A. Bunn Jr., L.E. Heasley, Expression of catalytically inactive phospholipase Cbeta disrupts phospholipase Cbeta and mitogen-activated protein kinase signaling and inhibits small cell lung cancer growth, Cancer Res. 58 (1998) 910–913.
[47] T. Seufferlein, E. Rozengurt, Galanin, neurotensin, and phorbol esters rapidly stimulate activation of mitogen-activated protein kinase in small cell lung cancer cells, Cancer Res. 56 (1996) 5758–5764.
[48] D.A. Jones, J. Cummings, S.P. Langdon, J.F. Smyth, Preclinical studies on the broad-spectrum neuropeptide growth factor antagonist G, Gen. Pharmacol. 28 (1997) 183–189.
[49] R. Paschke, M. Ludgate, The thyrotropin receptor in thyroid diseases, N. Engl. J. Med. 337 (1997) 1675–1681.
[50] T. Gudermann, F. Kalkbrenner, G. Schultz, Diversity and selectivity of receptor-G protein interaction, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 429–459.
[51] K.L. Laugwitz, A. Allgeier, S. Offermanns, K. Spicher, J. Van Sande, J.E. Dumont, G. Schultz, The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 116–120.
[52] G. Vassart, J.E. Dumont, The thyrotropin receptor and the regulation of thyrocyte function and growth, Endocr. Rev. 13 (1992) 596–611.
[53] C. O'Sullivan, C.M. Barton, S.L. Staddon, C.L. Brown, N.R. Lemoine, Activating point mutations of the gsp oncogene in human thyroid adenomas, Mol. Carcinog. 4 (1991) 345–349.
[54] H.G. Suarez, J.A. du Villard, B. Caillou, M. Schlumberger, C. Parmentier, R. Monier, gsp mutations in human thyroid tumours, Oncogene 6 (1991) 677–679.
[55] A. Shenker, L. Laue, S. Kosugi, J.J. Merendino Jr., T. Minegishi, G.B. Cutler Jr., A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty, Nature 365 (1993) 652–654.
[56] C. Voigt, H.P. Holzapfel, S. Meyer, R. Paschke, Increased expression of G-protein-coupled receptor kinases 3 and 4 in hyperfunctioning thyroid nodules, J. Endocrinol. 182 (2004) 173–182.
[57] P. Pujol, J.P. Daures, N. Nsakala, L. Baldet, J. Bringer, C. Jaffiol, Degree of thyrotropin suppression as a prognostic determinant in differentiated thyroid cancer, J. Clin. Endocrinol. Metab. 81 (1996) 4318–4323.
[58] C. Carmeci, D.A. Thompson, H.Z. Ring, U. Francke, R.J. Weigel, Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer, Genomics 45 (1997) 607–617.
[59] E.J. Filardo, J.A. Quinn, K.I. Bland, A.R. Frackelton Jr., Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF, Mol. Endocrinol. 14 (2000) 1649–1660.
[60] M. Maggiolini, A. Vivacqua, G. Fasanella, A.G. Recchia, D. Sisci, V. Pezzi, D. Montanaro, A.M. Musti, D. Picard, S. Ando, The G protein-coupled receptor GPR30 mediates c-*fos* up-regulation by 17beta-estradiol and phytoestrogens in breast cancer cells, J. Biol. Chem. 279 (2004) 27008–27016.
[61] P. Thomas, Y. Pang, E.J. Filardo, J. Dong, Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells, Endocrinology 146 (2005) 624–632.
[62] A.T. Porter, A.C.R.O. F., E. Ben-Josef, Humoral mechanisms in prostate cancer: A role for FSH, Urol. Oncol. 6 (2001) 131–138.
[63] Y. Daaka, Mitogenic action of LPA in prostate, Biochim. Biophys. Acta 1582 (2002) 265–269.
[64] J. Nelson, A. Bagnato, B. Battistini, P. Nisen, The endothelin axis: emerging role in cancer, Nat. Rev., Cancer 3 (2003) 110–116.
[65] A.L. Bookout, A.E. Finney, R. Guo, K. Peppel, W.J. Koch, Y. Daaka, Targeting Gbetagamma signaling to inhibit prostate tumor formation and growth, J. Biol. Chem. 278 (2003) 37569–37573.
[66] Y. Itoh, T. Joh, S. Tanida, M. Sasaki, H. Kataoka, K. Itoh, T. Oshima, N. Ogasawara, S. Togawa, T. Wada, H. Kubota, Y. Mori, H. Ohara, T. Nomura, S. Higashiyama, M. Itoh, IL-8 promotes cell proliferation and migration through metalloproteinase-cleavage proHB-EGF in human colon carcinoma cells, Cytokine 29 (2005) 275–282.
[67] A.B. Hendrich, K. Michalak, Lipids as a target for drugs modulating multidrug resistance of cancer cells, Curr. Drug Targets 4 (2003) 23–30.
[68] N. Mikirova, H.D. Riordan, J.A. Jackson, K. Wong, J.R. Miranda-Massari, M.J. Gonzalez, Erythrocyte membrane fatty acid composition in cancer patients, P. R. Health Sci. J. 23 (2004) 107–113.
[69] P.V. Escriba, A. Ozaita, C. Ribas, A. Miralles, E. Fodor, T. Farkas, J.A. Garcia-Sevilla, Role of lipid polymorphism in G protein–membrane interactions: nonlamellar-prone phospholipids and peripheral protein binding to membranes, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11375–11380.
[70] J. Martinez, O. Vogler, J. Casas, F. Barcelo, R. Alemany, J. Prades, T. Nagy, C. Baamonde, P.G. Kasprzyk, S. Teres, C. Saus, P.V. Escriba, Membrane structure modulation, protein kinase C alpha activation,

and anticancer activity of minerval, Mol. Pharmacol. 67 (2005) 531–540.
[71] Q. Yang, R. Alemany, J. Casas, K. Kitajka, S.M. Lanier, P.V. Escriba, Influence of the membrane lipid structure on signal processing via G protein-coupled receptors, Mol. Pharmacol. 68 (2005) 210–217.
[72] P.V. Escriba, Membrane-lipid therapy: a new approach in molecular medicine, Trends Mol. Med. 12 (2006) 34–43.
[73] J.A. Joseph, R. Cutler, G.S. Roth, Changes in G protein-mediated signal transduction in aging and Alzheimer's disease, Ann. N. Y. Acad. Sci. 695 (1993) 42–45.
[74] J. Pascual, C. del Arco, A.M. Gonzalez, A. Diaz, E. del Olmo, A. Pazos, Regionally specific age-dependent decline in alpha 2-adrenoceptors: an autoradiographic study in human brain, Neurosci. Lett. 133 (1991) 279–283.
[75] M. Sastre, J.A. Garcia-Sevilla, Density of alpha-2A adrenoceptors and Gi proteins in the human brain: ratio of high-affinity agonist sites to antagonist sites and effect of age, J. Pharmacol. Exp. Ther. 269 (1994) 1062–1072.
[76] M. Sastre, J. Guimon, J.A. Garcia-Sevilla, Relationships between beta- and alpha2-adrenoceptors and G coupling proteins in the human brain: effects of age and suicide, Brain Res. 898 (2001) 242–255.
[77] S. Mato, A. Pazos, Influence of age, postmortem delay and freezing storage period on cannabinoid receptor density and functionality in human brain, Neuropharmacology 46 (2004) 716–726.
[78] F. De Paermentier, S.C. Cheetham, M.R. Crompton, R.W. Horton, Beta-adrenoceptors in human brain labelled with [^{3}H]dihydroalprenolol and [^{3}H]CGP 12177, Eur. J. Pharmacol. 167 (1989) 397–405.
[79] N. Vijayashankar, H. Brody, A quantitative study of the pigmented neurons in the nuclei locus coeruleus and subcoeruleus in man as related to aging, J. Neuropathol. Exp. Neurol. 38 (1979) 490–497.
[80] M. Sastre, J.A. Garcia-Sevilla, Opposite age-dependent changes of alpha 2A-adrenoceptors and nonadrenoceptor [^{3}H]idazoxan binding sites (I_2-imidazoline sites) in the human brain: strong correlation of I_2 with monoamine oxidase-B sites, J. Neurochem. 61 (1993) 881–889.
[81] D.T. Jones, R.R. Reed, Molecular cloning of five GTP-binding protein cDNA species from rat olfactory neuroepithelium, J. Biol. Chem. 262 (1987) 14241–14249.
[82] W.F. Simonds, P.K. Goldsmith, J. Codina, C.G. Unson, A.M. Spiegel, Gi2 mediates alpha 2-adrenergic inhibition of adenylyl cyclase in platelet membranes: in situ identification with G alpha C-terminal antibodies, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 7809–7813.
[83] S. Marullo, L.J. Emorine, A.D. Strosberg, C. Delavier-Klutchko, Selective binding of ligands to beta 1, beta 2 or chimeric beta 1/beta 2-adrenergic receptors involves multiple subsites, EMBO J. 9 (1990) 1471–1476.
[84] L.T. Young, J.J. Warsh, P.P. Li, K.P. Siu, L. Becker, J. Gilbert, O. Hornykiewicz, S.J. Kish, Maturational and aging effects on guanine nucleotide binding protein immunoreactivity in human brain, Brain Res. Dev. Brain Res. 61 (1991) 243–248.
[85] R.F. Cowburn, J.O. Marcusson, A. Eriksson, B. Wiehager, C. O'Neill, Adenylyl cyclase activity and G-protein subunit levels in postmortem frontal cortex of suicide victims, Brain Res. 633 (1994) 297–304.
[86] S. Shimohama, H. Ninomiya, T. Saitoh, R.D. Terry, R. Fukunaga, T. Taniguchi, M. Fujiwara, J. Kimura, M. Kameyama, Changes in signal transduction in Alzheimer's disease, J. Neural Transm. Suppl. 30 (1990) 69–78.
[87] R.S. Jope, L. Song, X. Li, R. Powers, Impaired phosphoinositide hydrolysis in Alzheimer's disease brain, Neurobiol. Aging 15 (1994) 221–226.
[88] J.A. Garcia-Sevilla, M. Sastre, P.V. Escriba, Age-dependent increases of immunoreactive imidazoline receptors in the human brain: possible association of a 29/30 kDa protein with the I_2-imidazoline receptor identified by [^{3}H]idazoxan, Neurosci. Lett. 184 (1995) 133–136.
[89] F.M. Tranquilli Leali, M. Artico, S. Potenza, C. Cavallotti, Age-related changes of monoaminooxidases in rat cerebellar cortex, Eur. J. Histochem. 47 (2003) 81–86.
[90] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2001) 741–766.
[91] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[92] M.S. Forman, J.Q. Trojanowski, V.M. Lee, Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs, Nat. Med. 10 (2004) 1055–1063.
[93] F.M. LaFerla, S. Oddo, Alzheimer's disease: Abeta, tau and synaptic dysfunction, Trends Mol. Med. 11 (2005) 170–176.
[94] T.C. Saido, N. Iwata, Metabolism of amyloid beta peptide and pathogenesis of Alzheimer's disease. Towards presymptomatic diagnosis, prevention and therapy, Neurosci. Res. 54 (2006) 235–253.
[95] A. Kern, B. Roempp, K. Prager, J. Walter, C. Behl, Down-regulation of endogenous amyloid precursor protein processing due to cellular aging, J. Biol. Chem. 281 (2006) 2405–2413.
[96] T. Saito, N. Iwata, S. Tsubuki, Y. Takaki, J. Takano, S.M. Huang, T. Suemoto, M. Higuchi, T.C. Saido, Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation, Nat. Med. 11 (2005) 434–439.
[97] S. Howell, J. Nalbantoglu, P. Crine, Neutral endopeptidase can hydrolyze beta-amyloid(1–40) but shows no effect on beta-amyloid precursor protein metabolism, Peptides 16 (1995) 647–652.
[98] N. Iwata, Y. Takaki, S. Fukami, S. Tsubuki, T.C. Saido, Region-specific reduction of A beta-degrading endopeptidase, neprilysin, in mouse hippocampus upon aging, J. Neurosci. Res. 70 (2002) 493–500.
[99] K. Yasojima, H. Akiyama, E.G. McGeer, P.L. McGeer, Reduced neprilysin in high plaque areas of Alzheimer brain: a possible relationship to deficient degradation of beta-amyloid peptide, Neurosci. Lett. 297 (2001) 97–100.
[100] K. Yasojima, E.G. McGeer, P.L. McGeer, Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain, Brain Res. 919 (2001) 115–121.
[101] D.S. Wang, N. Iwata, E. Hama, T.C. Saido, D.W. Dickson, Oxidized neprilysin in aging and Alzheimer's disease brains, Biochem, Biophys. Res. Commun. 310 (2003) 236–241.
[102] N. Iwata, M. Higuchi, T.C. Saido, Metabolism of amyloid-beta peptide and Alzheimer's disease, Pharmacol. Ther. 108 (2005) 129–148.
[103] S.G. Anagnostaras, G.G. Murphy, S.E. Hamilton, S.L. Mitchell, N.P. Rahnama, N.M. Nathanson, A.J. Silva, Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice, Nat. Neurosci. 6 (2003) 51–58.
[104] G. Ferrari-DiLeo, D.D. Flynn, Diminished muscarinic receptor-stimulated [3H]-PIP2 hydrolysis in Alzheimer's disease, Life Sci. 53 (1993) PL439–PL444.
[105] P.T. Francis, A.M. Palmer, M. Snape, G.K. Wilcock, The cholinergic hypothesis of Alzheimer's disease: a review of progress, J. Neurol., Neurosurg. Psychiatry 66 (1999) 137–147.
[106] S.L. Minger, M.M. Esiri, B. McDonald, J. Keene, J. Carter, T. Hope, P.T. Francis, Cholinergic deficits contribute to behavioral disturbance in patients with dementia, Neurology 55 (2000) 1460–1467.
[107] M.K. Lai, O.F. Lai, J. Keene, M.M. Esiri, P.T. Francis, T. Hope, C.P. Chen, Psychosis of Alzheimer's disease is associated with elevated muscarinic M2 binding in the cortex, Neurology 57 (2001) 805–811.
[108] D.D. Flynn, D.A. Weinstein, D.C. Mash, Loss of high-affinity agonist binding to M1 muscarinic receptors in Alzheimer's disease: implications for the failure of cholinergic replacement therapies, Ann. Neurol. 29 (1991) 256–262.
[109] E. Masliah, G.M. Cole, L.A. Hansen, M. Mallory, T. Albright, R.D. Terry, T. Saitoh, Protein kinase C alteration is an early biochemical marker in Alzheimer's disease, J. Neurosci. 11 (1991) 2759–2767.
[110] R.S. Jope, Cholinergic muscarinic receptor signaling by the phosphoinositide signal transduction system in Alzheimer's disease, J. Alzheimers Dis. 1 (1999) 231–247.
[111] S.W. Tsang, M.K. Lai, S. Kirvell, P.T. Francis, M.M. Esiri, T. Hope, C.P. Chen, P.T. Wong, Impaired coupling of muscarinic M(1) receptors to G-proteins in the neocortex is associated with severity of dementia in Alzheimer's disease, Neurobiol. Aging 27 (2006) 1216–1223.
[112] T.G. Ohm, J. Bohl, B. Lemmer, Reduced basal and stimulated (isoprenaline, Gpp(NH)p, forskolin) adenylate cyclase activity in

Alzheimer's disease correlated with histopathological changes, Brain Res. 540 (1991) 229–236.
[113] R.F. Cowburn, C. O'Neill, R. Ravid, I. Alafuzoff, B. Winblad, C.J. Fowler, Adenylyl cyclase activity in postmortem human brain: evidence of altered G protein mediation in Alzheimer's disease, J. Neurochem. 58 (1992) 1409–1419.
[114] R.F. Cowburn, C. O'Neill, R. Ravid, B. Winblad, C.J. Fowler, Preservation of Gi-protein inhibited adenylyl cyclase activity in the brains of patients with Alzheimer's disease, Neurosci. Lett. 141 (1992) 16–20.
[115] A. Schnecko, K. Witte, J. Bohl, T. Ohm, B. Lemmer, Adenylyl cyclase activity in Alzheimer's disease brain: stimulatory and inhibitory signal transduction pathways are differently affected, Brain Res. 644 (1994) 291–296.
[116] E. Hashimoto, H. Ozawa, T. Saito, W. Gsell, N. Takahata, P. Riederer, L. Frolich, Impairment of G(salpha) function in human brain cortex of Alzheimer's disease: comparison with normal aging, J. Neural Transm. 111 (2004) 311–322.
[117] R.F. Cowburn, M. Vestling, C.J. Fowler, R. Ravid, B. Winblad, C. O'Neill, Disrupted beta 1-adrenoceptor-G protein coupling in the temporal cortex of patients with Alzheimer's disease, Neurosci. Lett. 155 (1993) 163–166.
[118] H.M. Huang, G.E. Gibson, Altered beta-adrenergic receptor-stimulated cAMP formation in cultured skin fibroblasts from Alzheimer donors, J. Biol. Chem. 268 (1993) 14616–14621.
[119] W.L. Bonkale, R.F. Cowburn, T.G. Ohm, N. Bogdanovic, J. Fastbom, A quantitative autoradiographic study of [^{3}H]cAMP binding to cytosolic and particulate protein kinase A in post-mortem brain staged for Alzheimer's disease neurofibrillary changes and amyloid deposits, Brain Res. 818 (1999) 383–396.
[120] M. Martinez, I. Mougan, Fatty acid composition of human brain phospholipids during normal development, J. Neurochem. 71 (1998) 2528–2533.
[121] J.D. Carver, V.J. Benford, B. Han, A.B. Cantor, The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects, Brain Res. Bull. 56 (2001) 79–85.
[122] M. Neuringer, W.E. Connor, n-3 fatty acids in the brain and retina: evidence for their essentiality, Nutr. Rev. 44 (1986) 285–294.
[123] M. Neuringer, W.E. Connor, D.S. Lin, L. Barstad, S. Luck, Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 4021–4025.
[124] P.E. Wainwright, H.C. Xing, L. Mutsaers, D. McCutcheon, D. Kyle, Arachidonic acid offsets the effects on mouse brain and behavior of a diet with a low (n-6):(n-3) ratio and very high levels of docosahexaenoic acid, J. Nutr. 127 (1997) 184–193.
[125] K.A. Youdim, A. Martin, J.A. Joseph, Essential fatty acids and the brain: possible health implications, Int. J. Dev. Neurosci. 18 (2000) 383–399.
[126] A. Walter, U. Korth, M. Hilgert, J. Hartmann, O. Weichel, K. Fassbender, A. Schmitt, J. Klein, Glycerophosphocholine is elevated in cerebrospinal fluid of Alzheimer patients, Neurobiol. Aging 25 (2004) 1299–1303.
[127] R.M. Nitsch, J.K. Blusztajn, A.G. Pittas, B.E. Slack, J.H. Growdon, R.J. Wurtman, Evidence for a membrane defect in Alzheimer disease brain, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 1671–1675.
[128] J.A. Conquer, M.C. Tierney, J. Zecevic, W.J. Bettger, R.H. Fisher, Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment, Lipids 35 (2000) 1305–1312.
[129] L. Ginsberg, J.H. Xuereb, N.L. Gershfeld, Membrane instability, plasmalogen content, and Alzheimer's Disease, J. Neurochem. 70 (1998) 2533–2538.
[130] E. Masliah, M. Mallory, N. Ge, M. Alford, I. Veinbergs, A.D. Roses, Neurodegeneration in the central nervous system of apoE-deficient mice, Exp. Neurol. 136 (1995) 107–122.
[131] M.S. Oitzl, M. Mulder, P.J. Lucassen, L.M. Havekes, J. Grootendorst, E.R. de Kloet, Severe learning deficits in apolipoprotein E-knockout mice in a water maze task, Brain Res. 752 (1997) 189–196.
[132] P. Krzywkowski, O. Ghribi, J. Gagne, C. Chabot, S. Kar, J. Rochford, G. Massicotte, J. Poirier, Cholinergic systems and long-term potentiation in memory-impaired apolipoprotein E-deficient mice, Neuroscience 92 (1999) 1273–1286.
[133] G.S. Roth, J.A. Joseph, R.P. Mason, Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging, Trends Neurosci. 18 (1995) 203–206.
[134] T. Crook, W. Petrie, C. Wells, D.C. Massari, Effects of phosphatidylserine in Alzheimer's disease, Psychopharmacol. Bull. 28 (1992) 61–66.
[135] L.G. Puskas, K. Kitajka, C. Nyakas, G. Barcelo-Coblijn, T. Farkas, Short-term administration of omega 3 fatty acids from fish oil results in increased transthyretin transcription in old rat hippocampus, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1580–1585.
[136] S.T. Henderson, High carbohydrate diets and Alzheimer's disease, Med. Hypotheses 62 (2004) 689–700.
[137] S. Kalmijn, L.J. Launer, A. Ott, J.C. Witteman, A. Hofman, M.M. Breteler, Dietary fat intake and the risk of incident dementia in the Rotterdam Study, Ann. Neurol. 42 (1997) 776–782.
[138] W.B. Grant, Dietary links to Alzheimer's disease: 1999 update, J. Alzheimer's Dis. 1 (1999) 197–201.
[139] M.A. Smith, G.J. Petot, G. Perry, Diet and oxidative stress: a novel synthesis of epidemiological data on Alzheimer's disease, J. Alzheimer's Dis. 1 (1999) 203–206.
[140] J.L. Cooper, Dietary lipids in the aetiology of Alzheimer's disease: implications for therapy, Drugs Aging 20 (2003) 399–418.
[141] M.R. Prasad, M.A. Lovell, M. Yatin, H. Dhillon, W.R. Markesbery, Regional membrane phospholipid alterations in Alzheimer's disease, Neurochem. Res. 23 (1998) 81–88.
[142] P.L. McGeer, E.G. McGeer, Inflammation, autotoxicity and Alzheimer disease, Neurobiol. Aging 22 (2001) 799–809.
[143] V. Solfrizzi, F. Panza, F. Torres, F. Mastroianni, A. Del Parigi, A. Venezia, A. Capurso, High monounsaturated fatty acids intake protects against age-related cognitive decline, Neurology 52 (1999) 1563–1569.
[144] F. Panza, V. Solfrizzi, A.M. Colacicco, A. D'Introno, C. Capurso, F. Torres, A. Del Parigi, S. Capurso, A. Capurso, Mediterranean diet and cognitive decline, Public Health Nutr. 7 (2004) 959–963.
[145] S. Acin, M.A. Navarro, R. Carnicer, J.M. Arbones-Mainar, M.A. Guzman, C. Arnal, G. Beltran, M. Uceda, N. Maeda, J. Osada, Dietary cholesterol suppresses the ability of olive oil to delay the development of atherosclerotic lesions in apolipoprotein E knockout mice, Atherosclerosis 182 (2005) 17–28.
[146] W.E. Schutzer, S.L. Mader, Age-related changes in vascular adrenergic signaling: clinical and mechanistic implications, Ageing Res. Rev. 2 (2003) 169–190.
[147] E.G. Lakatta, Cardiovascular regulatory mechanisms in advanced age, Physiol. Rev. 73 (1993) 413–467.
[148] B. Folkow, A. Svanborg, Physiology of cardiovascular aging, Physiol. Rev. 73 (1993) 725–764.
[149] K. Leineweber, T. Wangemann, C. Giessler, H. Bruck, S. Dhein, M. Kostelka, F.W. Mohr, R.E. Silber, O.E. Brodde, Age-dependent changes of cardiac neuronal noradrenaline reuptake transporter (uptake1) in the human heart, J. Am. Coll. Cardiol. 40 (2002) 1459–1465.
[150] D.R. Seals, M.D. Esler, Human ageing and the sympathoadrenal system, J. Physiol. 528 (2000) 407–417.
[151] O.E. Brodde, K. Leineweber, Autonomic receptor systems in the failing and aging human heart: similarities and differences, Eur. J. Pharmacol. 500 (2004) 167–176.
[152] J.R. Docherty, Cardiovascular responses in ageing: a review, Pharmacol. Rev. 42 (1990) 103–125.
[153] N. Ferrara, K. Davia, P. Abete, F. Rengo, S.E. Harding, Alterations in beta-adrenoceptor mechanisms in the aging heart, relationship with heart failure, Aging 9 (1997) 391–403.
[154] M. White, R. Roden, W. Minobe, M.F. Khan, P. Larrabee, M. Wollmering, J.D. Port, F. Anderson, D. Campbell, A.M. Feldman, et al., Age-related changes in beta-adrenergic neuroeffector systems in the human heart, Circulation 90 (1994) 1225–1238.
[155] O.E. Brodde, H.R. Zerkowski, D. Schranz, A. Broede-Sitz, M. Michel-Reher, E. Schafer-Beisenbusch, J.A. Piotrowski, H. Oelert, Age-dependent changes in the beta-adrenoceptor-G-protein(s)-adenylyl

cyclase system in human right atrium, J. Cardiovasc. Pharmacol. 26 (1995) 20–26.
[156] O.E. Brodde, U. Konschak, K. Becker, F. Ruter, U. Poller, J. Jakubetz, J. Radke, H.R. Zerkowski, Cardiac muscarinic receptors decrease with age. In vitro and in vivo studies, J. Clin. Invest. 101 (1998) 471–478.
[157] C. Giessler, T. Wangemann, H.R. Zerkowski, O.E. Brodde, Age-dependent decrease in the negative inotropic effect of carbachol on isolated human right atrium, Eur. J. Pharmacol. 357 (1998) 199–202.
[158] K. Leineweber, S. Klapproth, A. Beilfuss, R.E. Silber, G. Heusch, T. Philipp, O.E. Brodde, Unchanged G-protein-coupled receptor kinase activity in the aging human heart, J. Am. Coll. Cardiol. 42 (2003) 1487–1492.
[159] F. Roka, M. Freissmuth, C. Nanoff, G protein-dependent signalling and ageing, Exp. Gerontol. 35 (2000) 133–143.
[160] E.S. Werstiuk, R.M. Lee, Vascular beta-adrenoceptor function in hypertension and in ageing, Can. J. Physiol. Pharmacol. 78 (2000) 433–452.
[161] J. Bonnevier, R. Fassler, A.P. Somlyo, A.V. Somlyo, A. Arner, Modulation of Ca2+sensitivity by cyclic nucleotides in smooth muscle from protein kinase G-deficient mice, J. Biol. Chem. 279 (2004) 5146–5151.
[162] M.A. Gaballa, A.D. Eckhart, W.J. Koch, S. Goldman, Vascular beta-adrenergic receptor adenylyl cyclase system in maturation and aging, J. Mol. Cell Cardiol. 32 (2000) 1745–1755.
[163] W.E. Schutzer, J.F. Reed, M. Bliziotes, S.L. Mader, Upregulation of G protein-linked receptor kinases with advancing age in rat aorta, Am. J. Physiol., Regul. Integr. Comp. Physiol. 280 (2001) R897–R903.
[164] H. Gurdal, E. Friedman, M.D. Johnson, Beta-adrenoceptor-G alpha S coupling decreases with age in rat aorta, Mol. Pharmacol. 47 (1995) 772–778.
[165] W.E. Schutzer, V.J. Watts, J. Chapman, M.G. Cumbay, K.A. Neve, R.L. Neve, S.L. Mader, Viral-mediated gene delivery of constitutively activated G alpha s alters vasoreactivity, Clin. Exp. Pharmacol. Physiol. 27 (2000) 9–13.
[166] A. Benetos, F. Huguet, P. Albaladejo, A.M. Brisac, M. Pappo, M.E. Safar, B.I. Levy, Role of adrenergic tone in mechanical and functional properties of carotid artery during aging, Am. J. Physiol. 265 (1993) H1132–H1138.
[167] M.D. Johnson, Y. Zhou, E. Friedman, J. Roberts, Expression of G protein alpha subunits in the aging cardiovascular system, J. Gerontol., A, Biol. Sci. Med. Sci. 50A (1995) B14–B19.
[168] S.L. Mader, C.L. Downing, J. Amos-Landgraf, P. Swebjka, Age-related changes in G proteins in rat aorta, J. Gerontol., A, Biol. Sci. Med. Sci. 51 (1996) B111–B116.
[169] J. Chapman, M. Cohen-Armon, Y. Shoenfeld, A.D. Korczyn, Antiphospholipid antibodies permeabilize and depolarize brain synaptoneurosomes, Lupus 8 (1999) 127–133.
[170] B. Zheng, Y.C. Ma, R.S. Ostrom, C. Lavoie, G.N. Gill, P.A. Insel, X.Y. Huang, M.G. Farquhar, RGS-PX1, a GAP for GalphaS and sorting nexin in vesicular trafficking, Science 294 (2001) 1939–1942.
[171] J.D. Ollerenshaw, A.M. Heagerty, R.F. Bing, J.D. Swales, Abnormalities of erythrocyte membrane fatty acid composition in human essential hypertension, J. Hum. Hypertens. 1 (1987) 9–12.
[172] J. Villar, C. Montilla, O. Muniz-Grijalvo, F.G. Muriana, P. Stiefel, V. Ruiz-Gutierrez, J. Carneado, Erythrocyte $Na(^{+})$–Li^{+} countertransport in essential hypertension: correlation with membrane lipids levels, J. Hypertens. 14 (1996) 969–973.
[173] C. Russo, O. Olivieri, D. Girelli, P. Guarini, R. Pasqualini, M. Azzini, R. Corrocher, Increased membrane ratios of metabolite to precursor fatty acid in essential hypertension, Hypertension 29 (1997) 1058–1063.
[174] P. Mata, J.A. Garrido, J.M. Ordovas, E. Blazquez, L.A. Alvarez-Sala, M.J. Rubio, R. Alonso, M. de Oya, Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women, Am. J. Clin. Nutr. 56 (1992) 77–83.
[175] S. Heyden, Polyunsaturated and monounsaturated fatty acids in the diet to prevent coronary heart disease via cholesterol reduction, Ann. Nutr. Metab. 38 (1994) 117–122.
[176] V. Ruiz-Gutierrez, F.J. Muriana, A. Guerrero, A.M. Cert, J. Villar, Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources, J. Hypertens. 14 (1996) 1483–1490.
[177] F.J. Muriana, C. Montilla, J. Villar, V. Ruiz-Gutierrez, Transbilayer movement of erythrocyte membrane cholesterol in human essential hypertension, J. Hypertens. 13 (1995) 619–623.
[178] F.J. Muriana, A. Alonso, J. Villar, V. Ruiz-Gutierrez, Validity of studies on distribution and transbilayer movement of erythrocyte membrane cholesterol, J. Hypertens. 14 (1996) 1379–1380.
[179] F.J. Muriana, J. Villar, V. Ruiz-Gutierrez, Intake of olive oil can modulate the transbilayer movement of human erythrocyte membrane cholesterol, Cell Mol. Life Sci. 53 (1997) 496–500.
[180] V. Ruiz-Gutierrez, J.S. Perona, Y.M. Pacheco, F.J. Muriana, J. Villar, Incorporation of dietary triacylglycerols from olive oil and high-oleic sunflower oil into VLDL triacylglycerols of hypertensive patients, Eur. J. Clin. Nutr. 53 (1999) 687–693.
[181] J.S. Perona, J. Canizares, E. Montero, J.M. Sanchez-Dominguez, V. Ruiz-Gutierrez, Plasma lipid modifications in elderly people after administration of two virgin olive oils of the same variety (*Olea europaea* var. hojiblanca) with different triacylglycerol composition, Br. J. Nutr. 89 (2003) 819–826.
[182] P. North, S. Fleischer, Alteration of synaptic membrane cholesterol/phospholipid ratio using a lipid transfer protein. Effect on gamma-aminobutyric acid uptake, J. Biol. Chem. 258 (1983) 1242–1253.
[183] K. Tsuda, K. Kimura, I. Nishio, Y. Masuyama, Nitric oxide improves membrane fluidity of erythrocytes in essential hypertension: An electron paramagnetic resonance investigation, Biochem. Biophys. Res. Commun. 275 (2000) 946–954.
[184] M. Cimino, G. Vantini, S. Algeri, G. Curatola, C. Pezzoli, G. Stramentinoli, Age-related modification of dopaminergic and beta-Adrenergic receptor system: restoration to normal activity by modifying membrane fluidity with S-adenosylmethionine, Life Sci. 34 (1984) 2029–2039.
[185] J. Prades, S.S. Funari, P.V. Escriba, F. Barcelo, Effects of unsaturated fatty acids and triacylglycerols on phosphatidylethanolamine membrane structure, J. Lipid Res. 44 (2003) 1720–1727.
[186] R. Alemany, O. Vogler, S. Teres, C. Egea, C. Baamonde, F. Barcelo, C. Delgado, K.H. Jakobs, P.V. Escriba, Antihypertensive action of 2-hydroxyoleic acid in SHR mediated by enhancement of the protein kinase A pathway and downregulation of Rho-kinase, J. Lipid Res. 47 (2006) 1762–1770.
[187] N.M. Tsvetkova, I. Horvath, Z. Torok, W.F. Wolkers, Z. Balogi, N. Shigapova, L.M. Crowe, F. Tablin, E. Vierling, J.H. Crowe, L. Vigh, Small heat-shock proteins regulate membrane lipid polymorphism, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 13504–13509.