Review

Crosstalk between diabetes and brain: Glucagon-like peptide-1 mimetics as a promising therapy against neurodegeneration

CrossMark

A.I. Duarte [a,*], E. Candeias [a], S.C. Correia [a,b], R.X. Santos [a,b], C. Carvalho [a,b], S. Cardoso [a,b], A. Plácido [a,c], M.S. Santos [a,b], C.R. Oliveira [a,d], P.I. Moreira [a,e,**]

[a] Center for Neuroscience and Cell Biology, Life Sciences Department, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal
[b] Life Sciences Department, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal
[c] Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal
[d] Laboratory of Biochemistry, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal
[e] Laboratory of Physiology, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal

ARTICLE INFO

ABSTRACT

According to World Health Organization estimates, type 2 diabetes (T2D) is an epidemic (particularly in under development countries) and a socio-economic challenge. This is even more relevant since increasing evidence points T2D as a risk factor for Alzheimer's disease (AD), supporting the hypothesis that AD is a "type 3 diabetes" or "brain insulin resistant state". Despite the limited knowledge on the molecular mechanisms and the etiological complexity of both pathologies, evidence suggests that neurodegeneration/death underlying cognitive dysfunction (and ultimately dementia) upon long-term T2D may arise from a complex interplay between T2D and brain aging. Additionally, decreased brain insulin levels/signaling and glucose metabolism in both pathologies further suggests that an effective treatment strategy for one disorder may be also beneficial in the other. In this regard, one such promising strategy is a novel successful anti-T2D class of drugs, the glucagon-like peptide-1 (GLP-1) mimetics (e.g. exendin-4 or liraglutide), whose potential neuroprotective effects have been increasingly shown in the last years. In fact, several studies showed that, besides improving peripheral (and probably brain) insulin signaling, GLP-1 analogs minimize cell loss and possibly rescue cognitive decline in models of AD, Parkinson's (PD) or Huntington's disease. Interestingly, exendin-4 is undergoing clinical trials to test its potential as an anti-PD therapy. Herewith, we aim to integrate the available data on the metabolic and neuroprotective effects of GLP-1 mimetics in the central nervous system (CNS) with the complex crosstalk between T2D-AD, as well as their potential therapeutic value against T2D-associated cognitive dysfunction.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Diabetes mellitus is one of the most common metabolic diseases and a major disorder of insulin regulation that nowadays constitutes an epidemic [1–3]. In this regard, in 2004, Wild et al. [4] estimated that 171 million people worldwide (~3% of the population) were diabetic. More recent estimations pointed to 366 million diabetics in 2011, a number that will rise to 552 million by 2030 [5]. However, these numbers are somehow controversial, given the World Health Organization's prediction of ~300 million diabetics worldwide in 2025 [6]. Despite this controversy, what remains unquestionable is

that this pathology constitutes an important public health concern in 21st century, especially concerning type 2 diabetes (T2D).

Regarding T2D, estimates point to ~6% of adults worldwide affected by this disease, that also accounts for 90–95% of diabetes cases [3]. This scenario is further aggravated by the fact that T2D is becoming more common in young people, challenging the idea that it usually occurs in people over >30 years [7]. Moreover, the increased prevalence of T2D with aging, the co-occurrence of risk factors associated with modern lifestyle in developed and under development countries (e.g. increased longevity, obesity, sedentarism, hypertension) and the global crisis (with a negative impact in lifestyle and healthcare systems), currently renders this disease a heavy socio-economic burden [1,2]. Last, but not the least, chronic T2D and its long-term complications (e.g. peripheral and autonomic neuropathy, stroke, retinopathy, cardiovascular disease, renal failure) also have a significant impact in quality of life, as well as in morbidity and mortality of these patients [1,2].

Therefore, early diagnosis of T2D, improvement of glycemic control and a proactive prevention of its complications, together with efficient

---

* Corresponding author. Tel.: +351 239 820190; fax: + 351 239 822776.
** Correspondence to: P.I. Moreira, Institute of Physiology, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal. Tel.: +351 239 820190; fax: +351 239 822776.
E-mail addresses: anaimduarte@gmail.com (A.I. Duarte), pismoreira@gmail.com (P.I. Moreira).

therapeutic approaches, are of outmost importance to win the battle against this epidemic. The achievement of these goals will reduce substantially T2D financial impact, especially in western countries [1,2].

In this review, we will focus on the negative impact of chronic T2D in central nervous system (CNS), with a special emphasis on neurodegenerative diseases of the dementia-type (e.g. Alzheimer's disease (AD)), and the increasingly promising therapeutic potential of a new class of anti-T2D drugs, the incretin/glucagon-like peptide-1 (GLP-1) mimetics (particularly the most well-studied exendin-4), against T2D-associated neurodegeneration.

1.1. When long-term T2D crosstalks with brain function: a general overview on the affected molecular mechanisms

Although the etiology of type 1 diabetes (T1D) is well known and involves an absolute deficiency of insulin, T2D is a highly complex, multifactorial metabolic disease, characterized by a progressive β-cell failure (relative insulin deficiency), decreased insulin action and insulin resistance [2,7]. Additionally, T2D develops under a cluster of risk factors that includes high blood glucose, obesity, increased blood triacylglycerols and insulin resistance, which individually or collectively, also increase the risk for neurodegeneration/neuronal death, and functional and structural brain changes, culminating in cognitive dysfunction that underlies some dementia-type disorders (e.g. AD) [7,8].

Indeed, diabetes has been widely associated with slowly progressive brain damage [9,10] resulting in diabetic neuropathy and/or mild to moderately impaired cognitive function. Although both T1D and T2D patients seem to be affected by cognitive alterations, these are mostly clinically relevant in the elderly T2D [11]. Interestingly, despite the body weight gain often associated to T2D, cognitive dysfunction seems to correlate with a pronounced brain atrophy (particularly in cortical, subcortical and hippocampal areas) and, subsequently, decreased brain weight, as well as white matter abnormalities in these patients [12–20]. Noteworthy, T2D has been also described to influence the survival of AD patients and to increase the incidence of stroke, Parkinson's disease (PD) and several other neurological disorders [1,12,21].

Despite the limited knowledge on the molecular mechanisms underlying chronic damage in T2D CNS, it has been hypothesized that a complex interaction between this pathology and brain aging may occur, involving a multitude of mechanisms [1,11]. These include a defective insulin action, metabolic/mitochondrial dysfunction, oxidative stress, advanced glycation endproducts (AGEs) formation, increased aldose reductase activity, activated protein kinase C (PKC) and increased hexosamine pathway flux [1]. Herewith, we will briefly discuss the involvement of some of these mechanisms in the pathogenesis of long-term complications affecting T2D CNS.

1.1.1. Defective insulin action and its long-term consequences in T2D brain

Chronic peripheral hyperinsulinemia and insulin resistance are some of the main consequences of diabetes (especially T2D-related insulin resistance), leading to a downregulation of brain insulin uptake and its subsequent deprivation from the crucial beneficial effects of this hormone [22]. Indeed, the classic idea that CNS was insulin-insensitive and the hormone was unable to cross the blood–brain barrier (BBB), has been widely challenged in the last three decades [21,23,24], with accumulating evidence showing that insulin can reach high levels in brain [25,26] and exert long-term neuronal trophic effects [27].

It has been widely described that most of the insulin present in adult CNS derives primarily from pancreatic β-cells, being transported into the brain by the cerebrospinal fluid (CSF) and crossing the BBB via a carrier-mediated, saturable, regulatable, and temperature-sensitive active process. Therefore, it is not surprising that an acute peripheral hyperinsulinemia episode increases CSF insulin content [1,11,28]. On the other hand, chronic peripheral hyperinsulinemia (as in insulin

resistance or T2D) downregulates BBB insulin receptors (IR) and impairs brain insulin uptake [11]. Additionally, insulin resistance and glucose dysmetabolism in uncontrolled T2D may also decrease cerebral blood flow and oxidative glucose metabolism, probably via downregulation of neuronal insulin signaling (inhibition of IR phosphorylation) and glycolytic enzymes [1]. As a consequence, brain activity is rapidly affected (independently from any systemic effects), leading to progressively impaired learning, memory and cognition [29–31]. Similar results were observed in healthy individuals submitted to a hyperinsulinemic-euglycemic clamp [32] and in the streptozotocin (STZ)-induced T1D rat model, in which cognitive dysfunction was correlated with impaired hippocampal plasticity [33]. Additionally, Li et al. [34] suggested that impaired cognition could arise from impaired hippocampal insulin/insulin-like growth factor-1 (IGF-1) action and subsequent neuronal apoptosis. This hypothesis was further supported by the studies from Sima and Li [35] reporting that restoration of insulin signaling by the proinsulin C-peptide prevented those deleterious effects of T1D. However, a comparison of T2D versus T1D rats (BBZDR/Wor versus. BB/Wor rats, respectively) showed that neuronal loss, neurite degeneration, impaired amyloid precursor protein (APP) metabolism, increased hyperphosphorylated tau protein (P-Tau) and dysfunctional insulin/IGF-1 signaling were more severe in T2D, probably as a result of insulin resistance [36].

Besides insulin entry into the CNS via a carrier-mediated system, an alternative pathway involves its direct diffusion through the area postrema, a circumventricular region with a "leaky" BBB [37,38]. Furthermore, it has been also widely proposed that insulin can be synthesized de novo in brain (particularly in pyramidal neurons, e.g., from hippocampus, prefrontal cortex, entorhinal cortex, and olfactory bulb, but not in glial cells) [29], being then released by exocytosis [39]. This is consistent with reports that insulin is highly enriched in brain cortex, olfactory bulb, hippocampus, hypothalamus, and amygdala [40]. Herewith, insulin and IR-mediated signaling pathways (together with the inseparable IGF-1/IGF-1 receptor (IGF-1R)) seem to play a crucial role in the regulation of CNS metabolism, neuronal growth and differentiation, neuromodulation and neuroprotection [1].

Insulin is widely known as a peripheral regulator of glucose transport and metabolism [27,41,42], which involve a complex crosstalk between peripheral nutritional and other hormonal signals and specialized CNS neurons (the glucosensing neurons) from the hypothalamic arcuate nucleus. Besides expressing and responding to peripheral insulin and leptin (hormones that inform the brain regarding the peripheral metabolic and adiposity status), these glucosensing neurons were also shown to detect and respond to the very small decreases in glycemia that precede a meal in both humans and rodents [43]. And, depending on glucose levels, insulin can centrally regulate the ingestive behavior and energy homeostasis, via differential effects on glucosensing neuronal membrane potential [43,44], with low extracellular glucose levels promoting the activity of glucose-inhibited neurons (a subtype of glucosensing neurons), while high glucose levels increase the activity of the glucose-excited glucosensing neurons by closing their K^+_{ATP} channels [45]. Although the mechanisms involved herein are not completely understood, the anorexigenic effect of brain insulin signaling may be mediated by the activation of insulin receptor substrate (IRS)/ phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway in rat hypothalamic arcuate nucleus. As a result, K^+_{ATP} channels are activated and opened, probably due to 1) phosphatidylinositol 3,4,5-trisphosphate (PIP3) binding to K^+_{ATP} channel, lowering the ability of ATP to inhibit them, 2) a direct inhibition of ATP binding to the channel by PIP3, or 3) a PIP3-induced degradation of actin filaments around channels, a process in which Rho and Rac GTPases are likely candidates [46,47]. As K^+_{ATP} channels are opened, glucosensing neurons become hyperpolarized and inactive and neuronal firing is reduced, inhibiting the secretion of the orexigenic neuropeptide Y (NPY)/ agouti-related protein (AgRP) and decreasing food intake and body weight [46,47]. To further complicate this system, it has been

hypothesized that insulin may also hyperpolarize and inactivate the anorexigenic proopiomelanocortin (POMC) neurons, but as orexigenic NPY and AgRP cells are more potent, this will culminate in a net catabolic response [47]. Moreover, besides regulating body weight homeostasis, hypothalamic insulin signaling also controls peripheral glucose production and utilization, in a process that may also involve its action on CNS K^+_{ATP} channels [47]. More specifically, insulin-induced K^+_{ATP} channel activation at the level of mediobasal hypothalamus and subsequent activation of brain stem nuclei generates efferent vagal input to hepatocytes, inhibiting hepatic glucose output into bloodstream and decreasing blood glucose levels [48]. Once impaired, hypothalamic insulin signaling (and hypothalamus-liver axis) culminates in a decreased hepatic sensitivity to the circulating hormone and, subsequently, stimulates glucose production by the liver [46,49,50].

Interestingly, it has been suggested that impaired CNS insulin signaling arising from peripheral insulin resistance may have an orexigenic effect, increasing body weight [46]. In this regard, disruption of insulin receptor autophosphorylation and subsequent inhibition of IRS-1 and −2 phosphorylation and association with PI3K/Akt were described in the hypothalamus of obese rats [51]. Similarly, impaired brain insulin signaling associated to peripheral insulin resistance also leads to phosphorylation of IRS-1 at serine (Ser)/threonine (Thr) residues (instead of "traditional" tyrosine residues) by IRS kinases, blocking SH2 domain binding to IRS-1 and thereby inhibiting downstream insulin signaling and possibly constituting a negative feedback mechanism [52]. For instance, insulin resistance-mediated activation of c-Jun N-terminal kinase (JNK) promotes IRS-1 phosphorylation at Ser307 and, as this residue is adjacent to IRS-1 phosphotyrosine-binding (PTB, receptor-binding) domain, its phosphorylation hampers the interaction IR-IRS-1 and subsequent Tyr phosphorylation of IRS-1 [52], therefore limiting insulin action on body weight which, in turn, will be controlled by the orexigenic modulators NPY/AgRP and γ-aminobutyric acid (GABA), culminating in increased body weight [46,47,53]. Surprisingly, chronic insulin-induced phosphorylation of IRS-1 at Ser307 has been also proposed to mediate, at least partially, IRS-1 degradation and point also to a possible protective role for Ser phosphorylation [52]. However, data on this positive effect in the context of insulin resistance, diabetes or obesity are scarce and the underlying mechanisms elusive.

From the above-mentioned, it is evident that brain requires robust neuroendocrine counterregulatory mechanisms to maintain peripheral glucose levels within a narrow, non-deleterious range.

Although the involvement of CNS insulin in the control of peripheral glucose metabolism is becoming increasingly unraveled, its participation in the regulation of brain glucose metabolism is still a matter of debate. Indeed, after the traditional idea that brain glucose metabolism was essentially insulin insensitive [54], a hypothesis arose that it could be regulated by insulin only in glial cells [55–57]. More recently, it has been proposed that neuronal insulin/IR signaling cascades may control overall cerebral glucose metabolism [27,58–60]. Increasing evidence supports this hypothesis, namely: 1) the overlapping distributions of insulin, IR, and glucose transporters (GLUTs) isoforms 1 and 4 in selective brain regions (e.g., hippocampus and choroid plexus) [61], 2) modulation of cerebellar GLUT4 expression by changes in circulating insulin levels [62] and 3) brain regional changes in glucose utilization upon hyperinsulinemia in rodents [63]. However, the most unequivocal supporting evidence was that fasting insulin levels can maximally stimulate brain cortical glucose metabolism in humans, and that the insulin-induced increment in glucose uptake may involve the recruitment of the partially insulin-sensitive glial GLUT1 to the plasma membrane [64].

An alternative source of neuronal glucose seems to involve insulin-mediated inhibition of neuronal norepinephrine uptake, subsequent activation of glial β-adrenoreceptors and release of astrocytic glycogen that can be converted into glucose, which is then transported via the insulin-sensitive GLUT1 to the extracellular milieu for neuronal use [27,54,65]. Therefore, the crosstalk between CNS insulin levels/

action and glucose metabolism has to be tightly regulated, to avoid any deleterious changes that might affect energy production and neuronal survival [66]. Accordingly, we previously reported that insulin-induced neuronal IR/IGF-1R activation under oxidative stress and subsequent PI3K/Akt-mediated signaling prevented GLUT3 oxidation, restored glucose uptake and hexokinase-II expression, recovering neuronal glycolysis and energy levels [67,68]. Additionally, other authors suggested that insulin-induced antioxidant defenses in T1D could protect against GLUT oxidation and stimulate intracellular metabolism [69]. Nevertheless, there are also reports where insulin appears not to interfere with acute glucose transport into the brain [70,71], rendering this issue somehow still controversial.

Given the previously referred heterogenous distribution of brain IR, poor correlation between IR location and neuronal energy utilization, the insulin-independent neuronal glucose uptake, its neuromodulatory role in invertebrates, its action on neuronal norepinephrine and serotonin uptake, and its relation to NPY, it is not surprising that insulin may play other roles at CNS rather than metabolic regulation [27,61]. These include increased neurite outgrowth, regeneration of small myelinated fibers, maintenance of cortical, sympathetic and sensory neuronal survival during nervous system development, stimulation of neuronal protein synthesis, and improvement of synaptic activity and plasticity, memory formation, and storage, as well as neuroprotection [1,11].

In accordance with the above-mentioned studies regarding the negative effects of T2D-associated insulin resistance in CNS, insulin therapy has been shown to improve memory/learning in rats [46] and in healthy humans (upon intranasal administration), notably without changes in peripheral glycemia [32,72]. Additionally, systemic insulin infusion also improved verbal memory and attention [73]. And, in previous studies, we also showed that insulin restores synaptosomal GABA and glutamate uptake and extrasynaptosomal levels in rats submitted to oxidative stress and/or T2D [74,75]. These effects could be due to insulin's direct effect on neurotransmitter transport and/or to decreased ATP levels and subsequent reversal of the amino acid transporters under such conditions, thus protecting neurons against damaging effects of excitotoxicity or oxidative stress [74,75].

1.1.1.1. Impaired insulin signaling as the potential missing link between T2D and AD. Similarly to T2D, impaired insulin function has been also increasingly demonstrated in AD, suggesting that decreased brain insulin levels/action may constitute the missing link between both pathologies [1]. More specifically, several authors suggested that a prolonged peripheral hyperinsulinemia could impair the BBB and insulin transport into CNS, thus affecting IR activity and culminating in a brain insulin-resistance that could explain the lower insulin levels in CSF of AD patients [76,77]. Moreover, de la Monte and Wands [78] proposed that such disruption in brain insulin signaling could underlie the AD-associated neurodegeneration and cognitive decline. A hypothesis further supported by reports of: 1) decrement in insulin levels and IR expression in AD brain, 2) inverse correlation between AD Braak stage and levels of insulin, IGF-1 and −2 and their receptors, 3) decreased insulin clearance and increased amyloid-β (Aβ) levels in AD patients, 4) disrupted brain insulin function upon intracerebroventricular (icv) injection of STZ (a model of AD) that resulted in several features of human AD, and 5) memory restoration in AD patients upon insulin-glucose therapy [18]. Importantly, T2D-associated hyperinsulinemia has been also shown to increase Aβ peptide accumulation (a pathological hallmark of AD), due to the competition between insulin and Aβ for insulin degrading enzyme (IDE, a major Aβ-degrading enzyme) and its subsequent inhibition [79,80]. On the other hand, AD mice submitted to diet-induced insulin resistance and hyperinsulinemia, as well as the non-obese T2D Goto-Kakizaki (GK) rat brain, also showed increased Aβ accumulation and decreased IDE levels [81,82]. And similar results were described for AD patients with brain insulin resistance [83]. Studies from Querfurth

and LaFerla [84] reported that, besides reduced IDE expression, brains from patients with dementia and hyperglycemia also displayed hippocampal damage, increased P-Tau protein and glycogen synthase kinase-3β (GSK-3β) upregulation. Together with IDE-mediated Aβ degradation, insulin has been also shown to modulate γ-secretase activity and enhance Aβ trafficking from the endoplasmic reticulum and trans-Golgi network (the main sites for Aβ formation) to the plasma membrane, thereby increasing its extracellular levels [85,86].

Nevertheless, Kroner [87] proposed that, rather than hyperinsulinemia, it is the co-existence of brain insulin resistance and insulin deficiency that may underlie AD pathology. In this regard, AD could be faced as a brain-specific type of diabetes, the type 3 diabetes [87]. Interestingly, in a very recent study, Talbot et al. [88] showed that, despite no evident features of diabetes, AD brain is insulin and IGF-1 resistant, with dysfunctional IRS-1 mediating insulin resistance, whilst impaired IRS-2 underlying IGF-1 resistance. These authors also proposed that brain insulin resistance may be due (at least in part) to peripheral insulin resistance, reduced brain insulin uptake and increased brain Aβ oligomers. Conversely, brain IGF-1 resistance in AD (and subsequent impairment in IGF-1 signaling via IRS-2) could be an adaptive mechanism to delay Aβ accumulation and toxicity, as seen in animal models of AD [88].

Besides regulating Aβ levels, insulin also controls the other neuropathological hallmark of AD: P-Tau, thus reinforcing its role as the potential missing link between diabetes and AD [18,89]. Indeed, both peripheral hyperinsulinemia and CNS insulin resistance have been widely shown to modulate tau phosphorylation [18]. For example, Cheng et al. [90] reported that loss of IGF-1 signaling increased P-Tau and neurofibrillary tangles (NFTs) formation. But the more striking observations include the detection of NFTs in hippocampus of IRS-2-knockout mice (a model of T2D) [91] and increased P-Tau levels in NIRKO mice (a model with neuronal/brain-specific deletion of IR) [92]. However, given the different patterns of tau protein phosphorylation between the two animal models, the authors proposed that it could be mediated by both insulin resistance and hyperinsulinemia. Accordingly, Schubert et al. [92] observed that such increased P-Tau was due to the loss of insulin-mediated PI3K activation and subsequent impairment in the phosphorylation of both Akt and GSK-3β. Similarly, Jolivalt et al. [93] reported that a mouse model of T1D administered with insulin at the onset of the disease recovered learning skills and had less accumulated P-Tau in brain, probably due (at least partially) to the restoration of GSK-3β phosphorylation. More recently, the same authors demonstrated that impaired IR activity and increased GSK-3β were associated with an increment in P-Tau levels and in senile plaque formation in brains from APP transgenic mice with features of T1D [94]. Freude et al. [32] also found that peripherally-injected insulin directly targets the brain, rapidly initiating IR-mediated signaling pathways that culminate in site-specific tau protein phosphorylation. Similar results were previously obtained in cultured neurons treated with insulin or IGF-1 [95]. Therefore, results suggest that the increased incidence of AD in diabetic patients may also arise from the dysregulation of tau protein phosphorylation mechanisms under hyperglycemia and insulin resistance [18].

Of note is the fact that the mechanisms underlying the effects of T1D and T2D in AD pathology (and, more specifically, in P-Tau accumulation) may be different, with insulin deficiency playing the major role in increased P-Tau in T1D, while a cluster of hyperglycemia- plus insulin resistance-mediated increased P-Tau may be the major players in T2D [96]. However, Talbot et al. [88] suggested that tau pathology may not be essential for the development of brain insulin resistance in AD.

From the above-mentioned, it is not surprising that insulin has been increasingly faced as the potential missing link between chronic diabetes (particularly T2D) and dementia-type neurodegenerative diseases (as AD) [9,10], mostly due to its roles in the regulation of Aβ and tau protein "dynamics" in the brain. However, this hormone also plays a crucial role in several other brain functions, including cognition, memory and synaptic plasticity [97] (as previously discussed). Accordingly, insulin therapy

(at doses that do not affect hyperglycemia) has been shown to restore biochemical and pathological features of peripheral sensory neuropathy in animal models of T1D [59]. Besides this, insulin treatment also restores cognitive function in diabetic patients [50,98,99]. And, last but not the least, tissue (and cellular) dependence on insulin is such that its restriction under damaging conditions culminates in cell atrophy and apoptotic death [59]. In this regard, Soeda et al. [100] reported that mutating the SH2-containing inositol 5′-phosphatase 2 (SHIP2, a negative regulator of phosphatidylinositol 3,4,5-trisphosphate-mediated signals) in neurons promoted insulin signaling and ameliorated hippocampal synaptic plasticity and memory formation, while SHIP2 overexpression in mice impaired Akt-mediated IR/IGF-1R signaling and attenuated the neuroprotection afforded by insulin/IGF-1, culminating in neuronal apoptosis and memory impairment [100]. These authors also demonstrated that SHIP2 levels were increased in T2D *db/db* mice brain [100].

1.1.2. Impaired glucose metabolism/mitochondrial function in T2D brain

Together with decreased insulin action upon T2D, disruption of brain glucose metabolism (which may be also due to impaired glucose transport across BBB) seems to impair cerebral blood flow and oxidative glucose metabolism [30,101–103].

As extremely metabolically-active cells, neurons have high energy demands for synaptic transmission, axonal/dendritic transport, and ion channels/pump activity. Therefore, neuronal function and survival are highly affected by changes in brain glucose metabolism (including glucose supply, transport and utilization) [11,104]. This is further aggravated by neuronal inability to synthesize or store glucose, rendering them highly dependent on its transport across the BBB via GLUTs [105]. Thus, it is not surprising that increasing evidence points toward a correlation between insulin resistance, brain glucose dysmetabolism, synaptic function and memory deficits in both pre-diabetic and T2D [106,107]. Interestingly, we recently reported an increased accumulation of Aβ and P-Tau in sucrose-treated mouse brain (with metabolic alterations resembling those of T2D), further suggesting that (pre)diabetes might be a risk factor for AD [7]. Similar results were reported by others in both T1D and T2D rat brain, being more pronounced in T2D [36]. Taken together, these results favor the idea that amyloidogenesis (the transformation of a soluble protein into insoluble fibrillar protein aggregates) [108] could constitute a valid and reliable pathological hallmark to both AD and T2D [18].

In previous studies, we also reported a brain metabolic dysfunction in chronic T2D per se, given by the lower membrane potential and energy levels in adult GK rat brain synaptosomes [74]. In this regard, since 1) several metabolic partners are involved in neuronal energy supply and 2) neurons are extremely susceptible to changes in mitochondrial structure, localization and function that may limit energy production [18,109], mitochondria is increasingly recognized as a crucial participant in diabetes-related metabolic impairment and its long-term complications affecting CNS. As such, we recently reported that both sucrose-treated mice and 3xTg-AD mice (a model of AD) were highly enriched in swollen brain mitochondria, with disrupted mitochondrial membranes and cristae [7]. Similarly, Schmeichel et al. [110] reported structural abnormalities in mitochondria from both cultured dorsal root ganglion neurons submitted to high glucose levels or from chronically T1D STZ rats, with cultured neurons showing mitochondrial fragmentation/fission. Additionally, it has been suggested that an upregulation of DRP1 (a mitochondrial fission protein) at the initial phase could start a protective fission event that could be followed afterwards by the activation of Bim and Bax and apoptosis [111]. Surprisingly, Edwards et al. [112] showed recently that hyperglycemia could promote mitochondrial biogenesis in dorsal root ganglion from diabetic mice, probably resulting (at least partially) from the formation of small, fragmented mitochondria by fission. Accordingly, these authors also reported a hyperglycemia-induced increase in DRP1 levels and that inhibition of DRP1-induced fragmentation in vitro was neuroprotective against hyperglycemic damage [112].

Recently, a T1D-like insulin-deficient form of diabetes was demonstrated to be caused by inherited defects in mitochondrial DNA [113]. Moreover, our results in GK rat brain were (at least partially) explained by a dysfunctional mitochondrial electron transfer chain, further aggravated by aging and/or Aβ incubation in T2D [114]. Accordingly, in a very recent study, Carvalho et al. [7] observed that a dysfunctional respiratory chain and phosphorylation system in brain mitochondria from both sucrose-treated and 3xTg-AD mice underlined an ATP deficit, as also occurs in mitochondria from human AD platelets [115] and from brains (especially hippocampus and cortex) of other AD mouse models [116]. We also reported that brain mitochondria from chronically T1D rats (12 weeks after induction of diabetes with STZ), 3xTg-AD and sucrose-treated mice presented a lower capacity to accumulate and retain calcium (Ca^{2+}) [7,117]. And Mastrocola et al. [118] proposed that diabetes-induced nitrosylation or protein thiol stimulates brain mitochondrial nitric oxide synthase (NOS) activity, thus increasing nitric oxide (NO•) levels and inhibiting mitochondrial complexes III and IV and ATP synthase. As a result, ATP production is hampered and mitochondrial membrane potential collapses, leading to mitochondrial permeability transition pore (mPTP) opening and, ultimately, cell death [118]. The subsequent loss of synapses and synaptic function may in turn lead to cognitive decline [119]. Mitochondrial relevance in T2D is further emphasized by the involvement of human mitochondrial DNA (mtDNA) mutations and deletions of specific mitochondrial genes in dampening of oxidative phosphorylation in pancreatic β-cells from animal models and in diabetes onset [120].

Overall, these findings emphasize the role of mitochondrial dysfunction as a link between (pre)diabetes and AD. Nevertheless, things may not be as linear as expected, since Seaquist et al. [71] failed to observe changes in glucose transport upon short-term hyperglycemia.

1.1.3. Involvement of oxidative stress in T2D-mediated CNS lesion

Since mitochondria are also crucial in intracellular Ca^{2+} buffering and in the generation of reactive oxygen species (ROS), and the brain is highly susceptible to oxidative stress-induced damage [7], it is not surprising that T2D creates a deleterious vicious cycle in brain, involving metabolic/mitochondrial dysfunction and/or oxidative stress and, thus, chronically affecting CNS [109,114,121–123]. Interestingly, our previous studies reported an increase in plasma and brain vitamin E levels in adult T2D GK rats [124]. Additionally, the antioxidant coenzyme CoQ10 [121] and insulin [122] counteracted the deleterious effects of T2D and T1D in rat brain mitochondria, further highlighting the importance of metabolic/mitochondrial dysfunction and oxidative stress crosstalk in CNS. We also demonstrated a decrement in coenzyme CoQ9 levels in STZ rat brain mitochondria, suggesting that diabetes-related brain oxidative damage may also arise from impaired antioxidant mechanisms [122]. Similarly, Fernyhough et al. [125] described that increased ROS formation, lipid oxidation and protein nitrosylation in both T1D and T2D animal models were accompanied by decreased antioxidant defenses (e.g., glutathione and ascorbate). Therefore, Frisardi et al. [126] proposed that, similarly to Aβ, neurotoxicity induced by hyperglycemia may arise not only from high glucose levels, but also from the increased formation of reactive oxygen and nitrogen species and subsequent oxidative stress. This hypothesis was further supported by our recent observations that increased mitochondrial ROS production in sucrose-treated and 3xTg-AD mice brains was correlated with a decrease in antioxidant defenses [7]. More specifically, both mitochondrial aconitase (highly sensitive to superoxide [127] and inhibited upon neurodegeneration [128]) and glutathione reductase activities were decreased in both rodent models. Conversely, glutathione peroxidase activity was stimulated under these conditions. As a result, glutathione levels were reduced in these animals, in accordance with other studies [118,129,130]. The decrement in vitamin E levels in both AD and T2D mouse brain, and increased manganese superoxide dismutase activity were consistent with increased levels in hydrogen peroxide [7] and with other studies performed in plasma from diabetic [131] and AD

patients [132], as well as with evidence from the Tg2576 mouse model for AD [133]. Interestingly, this rise in mitochondrial oxidative stress in brains from both sucrose-treated and 3xTg-AD mice was correlated with an increased susceptibility to mPTP opening, as previously described by Kowaltowski et al. [134].

The involvement of oxidative stress in T2D-mediated brain damage is further complicated by the fact that it may also arise independently from metabolic/mitochondrial impairment. Nevertheless, the controversy remains on which appears first as disease progresses. Indeed, oxidative stress may originate from several other deleterious mechanisms associated with chronic hyperglycemia, including AGEs formation, glucose autoxidation, endoplasmic reticulum stress, and impaired antioxidant defenses, culminating in caspase-dependent neuronal death [135–137]. In this regard, it has been suggested that oxidative stress could be a major link in T1D-mediated apoptosis, since T1D animal models displayed increased hippocampal labeling with specific apoptotic markers (e.g. TUNEL staining, Fas and Bax expression, cytosolic apoptosis inducing factor (AIF), caspases-3 and −12 expression and activity) and 8-hydroxy-2′-deoxyguanosine (8-OHdG, a marker for oxidative stress) compared with T2D rats [136]. This was also correlated with a lower neuronal density in hippocampi from T1D than in T2D animals [136]. Similar pro-apoptotic effects were described by other authors in dorsal root ganglion neurons from diabetic rats [135,138].

1.1.4. Other pathophysiological mechanisms

1.1.4.1. AGEs/RAGE.
Other important biochemical players in this intricate puzzle are AGEs, whose abnormal formation and accumulation occur during normal brain aging, being accelerated by diabetes [139]. Indeed, studies reported an increased expression of the receptor for AGEs (RAGE) in neurons and glia from cognitively-affected diabetic mice [17]. Accordingly, interaction between AGEs and RAGE has been shown to promote ROS formation, widely described as an early damaging event in AD pathology [140]. Conversely, RAGE null diabetic mice were less susceptible to neurodegeneration than wild type diabetic mice [17]. Additionally, Girones et al. [141] observed that brain slices from post-mortem diabetic AD patients were more immunolabelled against AGEs than those from non-diabetic AD subjects and, more recently, Bruehl et al. [20] suggested that loss of hippocampal volume in T2D could be mediated by those AGEs. Furthermore, detection of AGEs in vascular walls, lipoproteins and lipid constituents were correlated with the development of macro- and microangiopathy and amyloidogenesis [142].

1.1.4.2. Impaired neurotransmission.
Given that glucose is also crucial for the brain maintenance of amino acid pool [143–145], it is not surprising that diabetes also impairs neurotransmission [146]. In fact, as previously discussed, neurotransmission is highly dependent on mitochondrial function, due to the massive energy-demanding mechanisms involved in neurotransmitter synthesis and removal [18,147,148]. Therefore, a crosstalk between mitochondrial dysfunction, oxidative stress and abnormal excitatory neurotransmitter release has been recently proposed to underlie the cognitive dysfunction reported in T1D patients under insulin therapy [146]. Accordingly, we and others previously showed a decrease in GABA levels in T1D STZ rat and in insulin-induced hypoglycemic cortical synaptosomes [146], as well as in plasma from T2D patients and individuals with impaired glucose tolerance [149]. This, together with increased Ca^{2+}-independent release in glutamate, suggests that neuronal dysfunction under hypoglycemia and/or untreated hyperglycemia in rats may also arise from a pathological release of excitatory cytoplasmic amino acids [146]. However, this was not mirrored in treated T2D rat brain synaptosomes, where exogenous insulin administration modulated GABA and/or glutamate transport under oxidative stress and/or T2D [74,75]. Additionally, others showed that hyperglycemia impaired glutamate release upon ischemia/

reperfusion in both non-diabetic [150] and diabetic animals [151,152], and that glutamate affinity for AMPA (but not NMDA receptors) was decreased in STZ rat brain [153]. Taurine and GABA transport was also shown to increase in diabetic rat retina and retinal pigment epithelium [154]. According to Guyot et al. [150], the increase in extracellular GABA and in the density of GABA_A receptors upon insulin treatment in T1D rats may constitute a protective mechanism against cytotoxic effects of released excitatory amino acids.

1.1.4.3. Decreased neurogenesis. From the above-mentioned, it is not surprising that diabetes compromises synaptic plasticity and learning potential. However, in the last years, it has been described that neurogenesis is also affected in both T1D [155–157] and T2D rodent models [158], as well as in hippocampus from high-fat fed rodents (a condition that accelerates diabetes) [159]. More specifically, Lang et al. [158] reported that, despite an initial increase in progenitor cell proliferation in the neurogenic regions of the adult GK rat brain, their survival was severely affected. According to the authors, this might constitute a feed-forward mechanism to increase proliferation in a vain attempt to maintain the steady-state formation of new neurons, thus compensating for the increased CNS cell death in the early phase of disease. But, as the animals' age, the long-term survival and the number of progenitor cells decrease in dentate gyrus [158]. Accordingly, if diabetes remains untreated, the newborn cells cannot survive and differentiate into fully functional neurons [160]. Although the mechanisms involved herein remain unclear, diabetes-associated chronic inflammation and oxidative stress may be involved [161–164]. Moreover, Lang et al. [158] proposed that unresponsiveness to growth factors may also play a role in this incapacity of newly formed cells to survive upon T2D. In this perspective, given the disruption of insulin signaling pathways in GK rats and the crosstalk between IGF-1 and IR, it is not surprising that their neural progenitors failed to respond to IGF-1, thus impairing PI3K/Akt/GSK-3β signaling, widely known to be involved in cell death [161]. Therefore, the declined learning and memory in diabetic patients may arise from hippocampal dysfunction [165], alternatively due to disruption of neurogenesis [158]. Accordingly, Oitzl et al. [166] showed that long-term exposure to high levels of corticosterone (as occurs in diabetes) impaired learning in animals, whilst maintenance of their physiological levels promoted neurogenesis, restored long-term potentiation (LTP) and reverted learning deficits upon T2D [156].

1.1.4.4. Recurrent hypoglycemic episodes. Evidences for chronic T2D damage to CNS mostly involve persistent hyperglycemia-associated lesion, leading to the idea that the simple normalization of blood glucose levels by insulin could protect against those injuries. However, we must bear in mind that cognitive dysfunction affecting these patients (as well as T1D individuals) may also arise from recurrent hypoglycemic episodes (the most common side-effect of insulin therapy) [1,167], as increasing evidence demonstrates that these episodes may adversely affect diabetic CNS [153,168]. For example, hypoglycemia was shown to affect endogenous levels of metabolites indirectly related with brain glucose metabolism, by accelerating lipolysis (increasing the formation of highly oxidizable polyunsatured fatty acids) and impairing protein synthesis, ion homeostasis, and mitochondrial function, leading to the neuronal dysfunction [54,169] that underlies motor incapacity, seizures, or cognitive damage, mainly in aged patients [170].

Despite the limited knowledge, hypoglycemia-related neuronal loss may play a crucial role in permanent diabetic brain damage [171]. Indeed, rather than the direct result of energy failure, such neuronal death appears to culminate in multiple deleterious events [172]. This further suggests that poor glycemic control may damage brain areas involved in learning and memory [173], thus limiting the analysis of insulin and hyperglycemia roles in diabetic brain [11].

Our group showed that, although insulin treatment was able to protect T1D STZ rat brain against oxidative stress and mitochondrial dysfunction (either after in vitro mitochondrial exposure to Aβ or

not) [117,122], this was not mirrored upon an acute episode of insulin-induced hypoglycemia in TD1 rat brain [173], in which increased levels of excitatory neurotransmitters (namely glutamine, glutamate, aspartate and taurine) and decreased levels of the inhibitory amino acid GABA were observed [146]. Accordingly, McGowan et al. [174] showed that acute episodes of hypoglycemia increase brain NMDA receptor binding sites to glutamate. Moreover, Battezzati et al. [175,176] described that, contrary to healthy individuals, T1D patients suffering a hypoglycemic–hyperinsulinemic clamp loose the counter-regulatory response that allows the utilization of excessively released glutamine as an alternative fuel to glucose, thus presenting abnormally high levels of this amino acid in plasma. Additionally, the lower release of GABA upon T1D and the acute hypoglycemia may further contribute to the severity of neuronal damage [146].

Overall, these results suggest that the increased capacity to release excitatory cytoplasmic amino acids and/or decreased release of inhibitory neurotransmitters upon diabetes and hypoglycemia could be related with increased oxidative stress-related neuronal membrane damage and cell death, as hypothesized by others [177–179]. Accordingly, a single acute insulin-induced hypoglycemic episode was shown to exacerbate the detrimental effects of T1D in brain cortical mitochondria bioenergetics and oxidative status [173].

1.2. Incretins/GLP-1 receptor mimetics

The high prevalence of both diabetes and AD in the increasingly aged population, together with the strong link between both pathologies and insulin signaling, fostered the recent search for efficient therapeutic/preventive strategies against diabetes-related AD, especially among currently Food and Drug Administration (FDA)-approved anti-diabetic drugs [180].

1.2.1. Previous anti-T2D drugs with promising neuroprotection features: what failed?

Amongst the several classes of anti-diabetic drugs, the use of sulphonylureas to treat T2D patients has declined in the last 20 years, mostly due to the more efficient combination of therapeutics that target the multitude of defects associated with the pathology [181–183]. Interestingly, the thiazolidinediones (TZD, from the class of anti-T2D drugs, the peroxisome proliferator-activated receptor-γ (PPARγ) agonists) rosiglitazone and pioglitazone are known to control neuroinflammation and oxidative stress and to activate STAT3- and Wnt-mediated signaling pathways. This could in turn provide the proper microenvironment for neural progenitor cells to proliferate and differentiate into mature neurons in diabetic brain [158,184]. Accordingly, these drugs were shown to decrease stroke-induced brain damage and neurological dysfunction in T2D mice [185]. Moreover, as an insulin sensitizer, rosiglitazone has been proposed to increase insulin signaling, which together with the decrease of insulin levels, might reduce its competition with Aβ for degradation by IDE, thus lowering Aβ$_{1-42}$ levels and improving learning and memory deficits [186]. However, rosiglitazone's and pioglitazone's side-effects in terms of cardiovascular risk led FDA to pose some restrictions for TZD prescription and use.

As previously described, insulin has been increasingly studied not only as an anti-T1D therapy, but also against T2D not controlled by diet, exercise or oral anti-diabetic agents, and it has been shown to decrease microvascular complications and mortality in these patients [187,188]. In fact, it has been increasingly suggested that all diabetic patients could be insulin treated, independently of disease progression [187]. Nevertheless, despite the great emphasis on diabetes care, healthy lifestyle and oral hypoglycemic therapy, as disease progresses, insulin's beneficial effects may be lost [189]. Therefore, in the later stages, the use of combined medications with synergistic effects and that address key pathophysiologic abnormalities of T2D without serious side-effects may have important advantages.

1.2.2. The novel and promising incretin/GLP-1 receptor mimetics in T2D CNS

Given the failure of other promising anti-T2D approaches, an increasingly used class of drugs is the incretin/glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists.

Incretins are gastrointestinal hormones, first identified in the 1960s, when an enteral glucose load increased insulin secretion greater than parenteral administration — the so-called "incretin effect" [190]. Initially, this difference was faced as secondary to an additional stimulus of gastrointestinal or hepatic origin [190]. Then, it was shown to result from the secretion of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) by enteroendocrine K and L cells, respectively [191], which account for ~70% of the insulin response to glucose [192].

Despite the decrement in GLP-1 secretion in T2D patients, its insulinotropic activity remains unaffected, while GIP presents an opposite pattern, possibly due to genetic factors [193,194]. Therefore, GLP-1 has been the primary research target for new T2D treatments based on incretin [192], namely the several GLP-1R agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors that are presently used clinically [189]. In this regard, exogenous administration of GLP-1 was shown to restore incretin physiology in T2D patients [195].

However, circulating GLP-1 has a short half-life, with up to 80% of it being degraded within 2 min after release by the enzyme DPP-4 [196,197]. DPP-4 is a ubiquitous aminopeptidase expressed in, e.g., liver, lung, kidney, endothelium and lymphocytes [191,198]. Besides incretins, DPP-4 has also other substrates, namely other gastrointestinal hormones, neuropeptides, cytokines and chemokines [191]. Therefore, several long-acting GLP-1R agonists that mimic the effects of native GLP-1 and that are more resistant to DPP-4-mediated degradation (with half-lives of 2.4 to 13 h that result in pharmacologic concentration of GLP-1) have been developed to treat T2D. Furthermore, DPP-4 inhibitors inactivate this enzyme and thus improve endogenous GLP-1 levels at the upper limit of the normal physiological range [191,199,200].

Although both GLP-1R agonists and DPP-4 inhibitors effectively achieved an optimal glucoregulation upon obesity, this required mostly a sustained receptor-mediated activation of the incretin axis (which also stimulated β-cell gene expression) [201]. Additionally, clinical studies comparing drugs from both classes demonstrated that exenatide or liraglutide (GLP-1R agonists used in T2D treatment [202]) were more efficient in lowering blood glucose levels upon hyperglycemia (but not euglycemia), glycated hemoglobin (HbA$_{1C}$) and body weight in T2D patients than sitagliptin (a DPP-4 inhibitor) [2,202,203]. And GLP-1R agonists were also more efficient in reducing total caloric intake [204]. Contrary to insulin and other oral anti-diabetic drugs, GLP-1R agonists also minimize the risk of hypoglycemia and may even improve β-cell function (an important issue, since at the time of T2D diagnosis, β-cell mass and function are already decreased) [2]. Nevertheless, in some patients exenatide and liraglutide also caused nausea and diarrhea [205,206].

1.2.2.1. The peripheral and brain GLP-1R.
GLP-1R are ubiquitously distributed throughout the organism, especially in pancreas (α- and β-cells), endothelium, heart, kidney, lung, brain, gastrointestinal tract and spinal cord [2,203,207–210]. Peripherally, once bound to its receptor, the endogenous GLP-1 (an incretin of 30 amino acid length) may exert multiple effects, including stimulation of glucose-dependent insulin secretion, and suppression of glucagon secretion, appetite and food intake and gastric emptying delay. And it also stimulates β-cell proliferation in preclinical models [203,211], as well as in both T1D and T2D patients [192,212–214]. As previously described, the most well-known role of incretins is their glucose-dependent insulinotropic effect, with insulin secretion decreasing as blood glucose levels become normal [2]. Despite the scarce and somehow controversial information, hampered GLP-1-mediated insulin secretion (a state of GLP-1 resistance) was detected in human subjects with diabetes, impaired glucose tolerance or insulin resistance [215–218], probably due to hyperglycemia per se and/or the genetic background [218]. Moreover, GLP-1 resistance may also arise from the decrement on the incretin content after a mixed meal in people suffering from T2D or impaired glucose tolerance [219,220], especially potentiated in the late-phase response [221]. However, other studies failed to report significant changes in fasting GLP-1 levels in humans with T2D or impaired glucose tolerance [193,222–225].

In CNS, GLP-1 expression occurs in the hypothalamus, cortex, hippocampus, striatum, substantia nigra, brain stem and subventricular zone (one of the neurogenesis areas in the adult brain) [207–209]. Under physiological conditions, GLP-1R expression is primarily confined to large output neurons, epitomized by pyramidal and dentate granule neurons, as well as Purkinje cells (where it localizes to dendrites and/or near synapses) [209]. Surprisingly, the detection of GLP-1R mRNA and protein in the brain of Zucker rats suggested that these receptors may be also synthesized in loco [226].

1.2.2.2. Functions of GLP-1R agonists in (diabetic) brain and periphery.
Besides the above-mentioned actions of GLP-1 mimetics, therapies involving these drugs were shown to decrease food intake and body weight, and to stimulate insulin secretion and gastric emptying more efficiently than DPP-4 inhibitors in rodent models [227], as well as in high fat-fed mice with glucose intolerance features [201].

Importantly, peripheral GLP-1 can cross the BBB by simple diffusion [228], gaining access to paraventricular nucleus and directly activating glucocorticoid formation without increasing glucose levels afterwards [229]. However, the mechanisms involved herein are not completely understood. Similarly, centrally-acting GLP-1 analogs (e.g. the GLP-1(7–36) amide) modulate food intake via neurons from the arcuate nucleus and circumventricular organs [226]. The earliest endocrine mechanism activated by food intake is the insulinotropic incretin effect and involves the secretion of GIP, followed immediately by that of the potent insulinotropic GLP-1 (both in T2D and non-diabetic individuals), culminating in increased blood insulin levels to deal with the post-prandial rise in glycemia [230]. However, it has been recently described that GLP-1 action in CNS is blunted in high-fat fed, insulin-resistant mice [231].

GLP-1R agonists were also able to enhance synaptic plasticity, strengthen LTP and improving cognitive performance [232–234]. This was further confirmed in mice overexpressing GLP-1R that showed improved learning skills [235], whilst knockout mice for GLP-1R had impaired memory formation [236].

Importantly, both peripherally-administered GLP-1 and its analogs have been reported to readily cross the BBB and reach the CNS [237,238], whereby they act as neurotrophic factors, inducing neurite outgrowth [239] and tyrosine hydroxylase expression [240,241], as well as anti-inflammatory [242–244] and as neuroprotective agents [245,246]. As described previously in this section, both peripherally- and centrally-administered GLP-1 analogs and agonists have potent, long-lasting anorexic effects (e.g., exenatide-induced weight loss was shown to persist for at least 2 years) [247–249]. Despite some controversy on the mechanisms involved herein, it has been hypothesized that nausea, decreased gastric emptying and increased satiety associated with GLP-1 mimetics could explain the decreased oral intake reported under such therapies [247]. Concerning the role of nausea, despite no significant correlation between nausea and decreased body weight were reported [247], several studies showed that individuals treated with exenatide or liraglutide who had nausea for several days also tended to lose more weight [248]. Alternatively, the GLP-1 agonist-associated ileal brake (and subsequent decreased gastric emptying) could also account for lowered food intake, probably via reduction of the initial postprandial increase in plasma glucose [250]. It is worth mentioning that ileal brake is the primary neuronal and hormonal inhibitory feedback mechanism that regulates the

transit of a meal via the gastrointestinal tract, thus optimizing nutrient digestion and absorption [247]. GLP-1 mimetics have been also described to promote satiety feelings, thus decreasing sensation of hunger and subsequent food intake [247].

From the above-mentioned, it is not difficult to understand that, if several questions remain pending on the physiological mechanisms by which GLP-1 mimetics inhibit food intake and decrease body weight, the subcellular/molecular mechanisms involved herein remain even less understood. In this regard, some evidence points towards a hypothalamic involvement (at least partially) in both CNS- and peripherally-administered GLP-1 decrease in caloric intake and body weight [247]. This hypothesis is supported by reports showing that: 1) GLP-1 synthesis occurs within the nucleus of the solitary tract neurons, which are known to innervate the hypothalamus [248], 2) injection of GLP-1 in the third ventricle promoted neuronal activation of paraventricular and arcuate nuclei of the hypothalamus, highly involved in energy balance regulation [248], and 3) GLP-1 and exenatide decreased ghrelin levels (a potent orexigenic hormone) [230] and may also act on other neuronal hypothalamic populations (e.g. POMC neurons) or crosstalk with other anorexigenic hormones (namely leptin), thus indirectly activating anorexic circuits [248]. However, the extent of the interaction between ghrelin and other satiety factors and weight loss remains unknown. Another striking evidence for such hypothalamic involvement in decreased food intake/body weight was that GLP-1 mimetics promoted hypothalamic neurogenesis, thereby increasing the number of healthy, GLP-1-responsive neurons involved in long-term glucose and body weight homeostasis [251].

Alternatively, body weight loss under GLP-1 treatment could be due to an increase in energy expenditure. In fact, Pannacciulli et al. [252] reported a correlation between higher fasting GLP-1 levels and higher resting energy expenditure and fat oxidation. Accordingly, GLP-1 infusion also promoted energy expenditure in healthy individuals [253], being abolished by constant insulin levels and suggesting an indirect effect on energy expenditure [248], probably mediated by a stimulation of sympathetic activity [254,255]. However, the mechanisms involved herein remain unknown and both human and rodent studies have produced conflicting results [248]. It is also worth mentioning the study by Perley and Kipnis [256] showing that, despite no changes in fat oxidation, a similar dose of GLP-1 infusion reduced carbohydrate oxidation and diet-induced thermogenesis.

As it is not our goal to extensively revise the role of all incretin-based therapies in this paper, we will focus mainly in the CNS effects of the most well-studied anti-T2D drugs of this class, exenatide (exendin-4) and liraglutide.

1.2.2.3. Exendin-4 in CNS. Exenatide is a synthetic injectable, DPP-4-resistant GLP-1R agonist derived from exendin-4, which is in turn a peptide derived from the saliva of the Gila monster, *Heloderma suspectum* [191,257]. Exendin-4 shares a 53% amino acid sequence identity with human GLP-1 [258] and the glucose-lowering potency of exenatide is ~5500-fold greater than that of endogenous GLP-1 [191,257].

Exendin-4 has been approved by the FDA in 2005, being indicated as an adjunct to diet and exercise to improve glycemic control in T2D adults [2], either as monotherapy or in combination with other oral anti-T2D agents (e.g. metformin, sulphonylureas and TZDs), but not with insulin [195]. Importantly, this GLP-1R agonist is stable in blood and most of the injected peptide immediately reaches the brain intact (rather than being trapped in BBB endothelial cells) [230,237]. This is not surprising, given exendin-4 high lipophilicity and independency from circumventricular organs to be transported into the CNS, being also insensitive to food deprivation for 24 h [237]. Once bound to GLP-1R (a 7-transmembrane protein that belongs to the class B1 G-protein-coupled receptor family) [259], exendin-4 (as GLP-1) initiates a signaling cascade that activates adenylyl cyclase, increasing cAMP levels (in a dose-dependent manner) which, in turn, interacts

with several downstream molecules, such as protein kinase A (PKA), PI3K and mitogen-activated protein kinase (MAPK) [203,260,261].

Recently, Li et al. [210] showed that PKA- and PI3K-mediated pathways are involved in the neurotrophic and neuroprotective roles of exendin-4 and GLP-1 in cultured neurons [240,241], while MAPK signaling plays a supportive role [239]. In 2000, Al-Barazanji et al. [262] showed that chronic exendin-4 therapy reduced body weight in both obese and lean Zucker rats by reducing food intake, suggesting a decrement in metabolic rate. Similar results were obtained upon direct injection of exendin-4 into the brain in non-obese animals [263,264]. More recently, Hayes et al. [260] described that such a decrease in food intake and body weight associated with exendin-4 was mediated by PKA and MAPK signaling. More specifically, these effects of exendin-4 were possible due to the coordinated PKA-induced suppression of AMP-activated protein kinase (AMPK) and p70S6 kinase (p70S6K), as well as to the activation of MAPK in hindbrain, thus representing a possible efficient anti-obesity approach [260]. On the other hand, MAPK-independent pathways and growth factor-dependent Ser/threonine (Thr) Akt have been also described [245], as well as an Epac-mediated pathway [265]. In this perspective, the differentially-modified GLP-1 signaling in different models of dietary obesity may explain the conflicting results described in obese humans [266–269], with some studies reporting a suppression of food intake in lean and obese humans upon GLP-1 administration [270,271]. Additionally, a central GLP-1-mediated neuronal activation of brainstem and hypothalamic structures potentially involved in conveying satiety signals may be essential to attenuate the perceived orosensory reward of palatable food and reduce food intake initiated by a meal [263,264]. As a result, excessive consumption of high dietary fat may impair the anorexigenic gut–brain feedback, stimulating the feeling of reward upon the intake of sweetened taste food [263]. In this regard, successful anti-obesity therapies may involve an inhibition of the desire to overconsume palatable and obesogenic foods (e.g. by improving hypothalamic neuronal recognition of increased glycemia after a meal, thereby accelerating the state of fullness), as well as re-establishment of anorexigenic signals [263,226] and activation of the enteric neurons [272]. On one hand, metabolites arising from hypothalamic neuronal glucose oxidation may be involved in satiety-related signaling pathways; on the other hand, hypothalamic receptors may control energy expenditure via modulation of CNS and peripheral action of leptin, insulin, glucocorticoids, galanin or NPY [226].

Several authors observed that subcutaneous exendin-4 administration also decreased the hypothalamic content of serotonin and norepinephrine in obese and lean rats, two neurotransmitters involved in the regulation of food intake [226,263]. Recently, Darsalia et al. [273] hypothesized that exendin-4 neuroprotection may occur via either GLP-1R-dependent or –independent pathways, in a dose-dependent manner.

Similarly to native GLP-1, peripheral exenatide was shown to stimulate glucose-dependent, first- and second-phase insulin secretion [274], inhibit glucagon secretion, slow gastric emptying, and reduce appetite/caloric intake, thus promoting weight loss [275]. Conversely, recent evidence showed that the anti-stroke efficacy of GLP-1R activation in non-diabetic mice was not accompanied by changes in insulin secretion, suggesting that its CNS effects are not due to changes in glycemia [276].

In preclinical and clinical studies exenatide was also demonstrated to promote cellular neurogenesis and proliferation, and to inhibit apoptotic death, increasing β-cell mass [191,277] and function [274,278]. Importantly, the anti-apoptotic effect of exendin-4 described in clonal insulinoma (INS-1E) cell lines treated with human islet amyloid polypeptide (hIAPP) was mediated (at least partially) by Akt pathway, since the peptide rescued treated cells from hIAPP-induced FOXO1, inhibition of pdx-1 translocation into the nucleus and activation of caspase-3 (known mediators of apoptotic cell death), recovering cell mass [279]. Furthermore, as Akt signaling has

been intimately coupled with mitochondrial biogenesis, it is not surprising that exendin-4 also restored the expression of mitochondrial DNA-encoded genes, number of mitochondria and activity of succinate dehydrogenase (indicative of improved mitochondrial function) under such damaging conditions [279]. Other clinical studies reported a long-lasting exenatide-mediated HbA$_{1c}$ and post-prandial glucose lowering in T2D patients, with some effects maintained for 3 years [195,278,280]. Similar exendin-4-mediated reduction in glycemia was reported in T2D GK rats [273].

Similarly to the anti-apoptotic effect of exendin-4 on pancreatic β-cells, GLP-1 analogs also protected cultured neurons against oxidative stress-, hypoxia- and trophic factor withdrawal-associated apoptosis [239,240,281]. Accordingly, in vivo studies reported a reduction in brain damage and improved functional outcome upon treatment with GLP-1 analogs after stroke [240,276], as well as in several animal models of PD [240,243,282,283] and Huntington's disease (HD) [246], peripheral neuropathy [284] and in AD alone [281,285–287] or in association with T2D [261]. In fact, exendin-4 is already being assessed as a new therapeutic strategy against PD and AD.

Importantly, those neuroprotective effects of GLP-1 analogs were also accompanied by increased neurogenesis, as given by the increase in neuronal progenitor cells in the brain of GLP-1 agonist-treated animals, in the number of surviving neurons in stroke-damaged striatum and cortex and in increased neurogenesis in an experimental model of PD [234,273,282,283,285]. In a very recent study, Li et al. [210] observed that exendin-4 has neuroprotective and neurotrophic properties in the superoxide dismutase-1 (SOD1) models (both cellular and animal models) of amyotrophic lateral sclerosis (ALS). These beneficial effects for motor neurons included glucose tolerance amelioration, preservation of lumbar spinal cord structure and neuron density, restoration of specific spinal cord markers and running behavior, as well as a reduction in apoptosis in spinal cord and in astrocytosis/microglia activation (at both presymptomatic and end stage disease, thus decreasing proinflammatory cytokine production) [210,242]. Additionally, exendin-4 protected neuronal cell lines against oxidative stress [180,288], ameliorated staurosporine-induced apoptosis (known to be mediated by active caspase-3 and increased Bax expression) upon trophic factor withdrawal [210,240,241], increased tyrosine hydroxylase expression (a dopaminergic marker) in dopaminergic neurons [240], as well as a cholinergic marker (choline acetyltransferase) and cell viability [210]. Exendin-4 was also neuroprotective against glutamate excitotoxicity [286]. Interestingly, its protective effect against high glucose levels was mediated by the PI3K pathway in PC12 cells [180]. Exendin-4 also reduced the in vitro generation of Aβ in cultured neurons under both euglycemic and hyperglycemic conditions and in mouse brain, protected against Aβ-associated hippocampal neuronal death and rescued learning and memory performance in ICV-STZ rats (a model of sporadic AD) [180,261,281,287]. These effects were correlated with protection against hippocampal degeneration and P-Tau, probably via downregulation of GSK-3β [180,246,281,287].

Surprisingly, Kim et al. [261] reported that exendin-4 was able to fully reverse the massive increase in soluble Aβ levels in the brains of 3xTg-AD female mice injected with STZ. Similarly, the neuroprotective effect of exendin-4 was recently described also in STZ rodents (which have been also associated with AD due to increased brain levels of Aβ) [281]. Given these results and that female gender is a well-known risk factor for AD, exendin-4 offers a promising therapeutic avenue against this pathology, providing preclinical support for translational studies in diabetic and/or AD patients [261].

Interestingly, given its role in body weight reduction, the protective effect of exendin-4 was also extended to high fat-fed mice (which are also more prone to T2D). Herein, the GLP-1R agonist was able to improve metabolic control, learning and memory, rescue LTP and hippocampal synaptic plasticity, thus improving cognitive function upon obesity [289], and may thus constitute a promising therapy against obesity.

Concerning anti-hyperglycemic agents, the adverse effects (e.g. gastrointestinal upset, hypoglycemia and weight gain) associated with their use can limit successful glycemic management, but are mostly transient, mild to moderate and prevalent at the treatment initiation [195,290]. Moreover, a once weekly formulation of exenatide is in clinical development and involves its encapsulation within biodegradable microspheres of poly(D,L-lactic-co-glycolic acid), to allow for gradual and controlled drug delivery [291], thus representing a huge advantage compared to the current twice daily regimen [292,293]. Ongoing studies revealed promising results of this new formulation for exenatide therapy [2].

1.2.2.4. Liraglutide in CNS. Another recently approved anti-T2D GLP-1R agonist therapy is liraglutide. This has the advantage of only requiring a once daily administration to improve glycemic control in T2D adults, together with diet and exercise [2]. Similarly to exenatide, liraglutide can be used as monotherapy or in combination with metformin, a sulphonylurea or a TZD to treat T2D, but not with insulin [2].

Liraglutide was formed by the replacement of lysine 34 of human GLP-1 with arginine, and by the attachment of a C16 fatty acid chain at position 26, using a γ-glutamic acid spacer, thus maintaining ~97% of the native GLP-1 sequence. This also facilitates albumin-binding and DPP-4 resistance, thereby allowing a half-life of ~13 h and the single daily dosage described [294]. Similarly to native GLP-1 and exenatide, liraglutide binding to GLP-1R activates adenylyl cyclase and upregulates cAMP/PKA signaling pathways [295], promoting the cAMP response element-binding (CREB) phosphorylation and nuclear gene transcription [296]. As a GLP-1R agonist, "traditional" liraglutide functions include stimulation of glucose-dependent insulin secretion, weight loss, satiety (decreasing food intake) and slowing gastric emptying [294]. Therefore, and similarly to exendin-4, liraglutide has been also proposed to be efficient in obesity treatment, in part due to its resistance to DPP-4-mediated degradation and subsequent longer half-life (and functional activity) [232,260]. Interestingly, liraglutide was shown to be more effective than exenatide in decreasing HbA$_{1c}$, with less nausea and hypoglycemia episodes. Nevertheless, side-effects were more severe with liraglutide than exenatide [2].

Concerning the role of liraglutide in CNS, available knowledge remains limited. At this respect, some authors described that, similarly to exendin-4, liraglutide also increased proliferation of progenitor cells in the subgranular zone of the dentate gyrus in high fat-fed mice (a model of T2D), as well as in non-obese mice [160,297]. This increase in new neurons is of the outmost interest, as it may have potential beneficial regenerative effects in neurodegenerative diseases, such as AD [298,299]. In other study, peripherally-injected liraglutide was also able to decrease plaque formation, rescue memory and cognitive performance, promote hippocampal synaptic plasticity and decrease inflammation in high fat-fed mice and in the presenilin 1 (APP/PS1) mouse model of AD [160,285,300], similarly to other reports [289,300]. Others demonstrated recently that intrahippocampal injection of liraglutide in rats also protects against Aβ$_{25–35}$-induced impairment in learning and memory, in a dose-dependent manner, supporting the idea that liraglutide could be probably an important player in the prevention or treatment of memory loss in AD patients [296]. However, clinical trials involving AD patients are essential to test this hypothesis. Interestingly, these authors also reported that pretreatment with liraglutide prevented Aβ$_{25–35}$-induced impairment of LTP, probably via a rapid and transient upregulation of intracellular cAMP levels (as occurs in astrocytes and hypothalamic neurons [242,251]), and subsequent cAMP/PKA signaling pathways, thereby improving learning and memory. These results were consistent with others obtained with lixisenatide [297], another GLP-1R agonist that will be released soon onto the market [301]. Alternatively, GLP-1R stimulation could also activate growth factor-like signaling cascade [232], increase dendritic sprouting and neuronal regeneration

and prevent or reduce damage associated with chronic exposure to Aβ [245,287].

In a recently published study, Porter et al. [302] showed that liraglutide also rescued LTP, plasma glucose, insulin levels, food intake in adult obese diabetic (*ob/ob*) mice (that also suffer from insulin resistance). According to these authors, liraglutide's beneficial effects on metabolic control and synaptic plasticity appear to be at least partially-mediated by increased Mash1 expression, which has been also suggested to improve hippocampal neurogenesis and cell survival in these mice.

As exendin-4, peripheral liraglutide also suppressed food intake, via activation of GLP-1R from vagal afferents, as well as via CNS-mediated effects [303], supporting the idea that this peptide may cross BBB. Indeed, in a very recent study, Hunter and Holscher [297] demonstrated that both liraglutide and lixisenatide were able to cross the BBB, in a highly regulated and time- and dose-dependent fashion, thereby activating CNC GLP-1R. According to these authors, such transport may shutdown upon supraphysiologic doses. However, the dose of lixisenatide required for this transport and to activate GLP-1R appears to be lower than that of liraglutide [297].

Importantly, exendin-4 is currently on the market as a T2D treatment and is injected peripherally twice-daily, while liraglutide is injected peripherally once daily [304]. Lixisenatide is under development to be used as a once-daily treatment for T2D [301]. Interestingly, these drugs can be also taken by non-diabetic people, as they only affect blood sugar levels in a hyperglycemia situation [305].

2. Conclusion

In the last years, the T2D epidemic emerged as an increasingly social and economic challenge, mostly in under development countries. Despite the etiological complexity of T2D (involving a cluster of several risk factors, mostly associated with modern lifestyle) and the limited knowledge on the molecular mechanisms involved herein, it has been widely proposed that neurodegeneration/cell death associated with chronic T2D may result from a complex interaction between the pathology and brain aging. As a result, brain structural and functional changes may arise, culminating in cognitive dysfunction underlying dementia-type disorders (e.g. AD) (Fig. 1). Indeed, AD has been hypothesized as the "type 3 diabetes" affecting the brain.

In summary, as the success against long-term complications of T2D rely mostly on early diagnosis, healthy lifestyle and efficient therapies, given the success of several in vitro and in vivo studies using GLP-1 mimetics, it is tempting to hypothesize that, together with improvement of insulin secretion, these GLP-1 analogs may overcome an increasingly recognized GLP-1-resistant state occurring in T2D, obesity or AD, thus constituting a potential therapy against cognitive dysfunction occurring under these circumstances (Fig. 1). However, the results from the ongoing clinical trials (and further basic research) will be essential to improve knowledge on this subject.

Fig. 1. Molecular mechanisms underlying long-term T2D in CNS and AD. Increasing evidence demonstrates that T2D is a risk factor for AD and that AD patients are more prone to T2D. Although the molecular mechanisms involved herein are not completely clarified, it has been suggested that a complex interaction between T2D and brain aging may occur, involving e.g. a decrease in brain insulin (Ins) levels and/or action (Ins receptor, IR), an impairment in brain glucose uptake and metabolism (despite peripheral hyperglycemia), mitochondrial dysfunction and oxidative stress (ROS). Increased advanced glycation endproducts (AGEs) formation and interaction with their receptors (RAGE) and impaired neurotransmission may be also involved, decreasing neurogenesis and promoting neurodegeneration/death, which underlie brain atrophy and cognitive dysfunction seen in both pathologies. Interestingly, GLP-1 mimetics have been recently demonstrated to exert neuroprotective/neurogenesis effects against deleterious conditions (e.g. AD and PD models), besides their well-known insulinotropic and anorexigenic properties.

References

[1] A.I. Duarte, P.I. Moreira, C.R. Oliveira, Insulin in central nervous system: more than just a peripheral hormone, J. Aging Res. (2012) 384017–384038.
[2] R.K. Campbell, Clarifying the role of incretin-based therapies in the treatment of type 2 diabetes mellitus, Clin. Ther. 33 (2011) 511–527.
[3] R. Sicree, J. Shaw, P. Zimmet, in: D. Gan (Ed.), Diabetes Atlas, third ed., International Diabetes Federation, Brussels, 2006, pp. 15–103.
[4] S. Wild, G. Roglic, A. Green, R. Sicree, H. King, Global prevalence of diabetes: estimates for the year 2000 and projections for 2030, Diabetes Care 27 (2004) 1047–1053.
[5] International Diabetes Federation, Diabetes Atlas: Diabetes and impaired glucose tolerance. Global burden: prevalence and projections, 2010 and 2030, http://www.idf.org/diabetesatlas/5e/the-global-burden2012, (Accessed August 2nd, 2012).
[6] World Health Organization, Diabetes facts, fact sheet no. 312, http://www.who.int/mediacentre/factsheets/fs312/en/2011, (accessed August 2nd, 2012).
[7] C. Carvalho, S. Cardoso, S.C. Correia, R.X. Santos, M.S. Santos, I. Baldeiras, C.R. Oliveira, P.I. Moreira, Metabolic alterations induced by sucrose intake and Alzheimer's disease promote similar brain mitochondrial abnormalities, Diabetes 61 (2012) 1234–1242.
[8] J.A. Luchsinger, C. Reitz, B. Patel, M.X. Tang, J.J. Manly, R. Mayeux, Relation of diabetes to mild cognitive impairment, Arch. Neurol. 64 (2007) 570–575.
[9] L. Li, C. Hölscher, Common pathological processes in Alzheimer disease and type 2 diabetes: a review, Brain Res. Rev. 56 (2007) 384–402.
[10] G.J. Biessels, W.H. Gispen, The impact of diabetes on cognition: what can be learned from rodent models? Neurobiol. Aging 26 (2005) S36–S41.
[11] P.I. Moreira, A.I. Duarte, M.S. Santos, A.C. Rego, C.R. Oliveira, An integrative view of the role of oxidative stress, mitochondria and insulin in Alzheimer's disease, J. Alzheimers Dis. 16 (2009) 741–761.
[12] R. Magierski, I. Kłoszewska, T.M. Sobów, The influence of vascular risk factors on the survival rate of patients with dementia with Lewy bodies and Alzheimer disease, Neurol. Neurochir. Pol. 44 (2010) 139–147.
[13] T. Akisaki, T. Sakurai, T. Takata, H. Umegaki, A. Araki, S. Mizuno, S. Tanaka, Y. Ohashi, A. Iguchi, K. Yokono, H. Ito, Cognitive dysfunction associates with white matter hyperintensities and subcortical atrophy on magnetic resonance imaging of the elderly diabetes mellitus Japanese elderly diabetes intervention trial (J-EDIT), Diabetes Metab. Res. Rev. 22 (2006) 376–384.
[14] T. den Heijer, S.E. Vermeer, E.J. van Dijk, N.D. Prins, P.J. Koudstaal, A. Hofman, M.M. Breteler, Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI, Diabetologia 46 (2003) 1604–1610.
[15] S.M. Manschot, A.M. Brands, J. van der Grond, R.P. Kessels, A. Algra, L.J. Kappelle, G.J. Biessels, Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes, Diabetes 55 (2006) 1106–1113.
[16] R. Schmidt, L.J. Launer, L.G. Nilsson, A. Pajak, S. Sans, K. Berger, M.M. Breteler, M. de Ridder, C. Dufouil, P. Fuhrer, S. Giampaoli, A. Hofman, Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study, Diabetes 53 (2004) 687–692.
[17] C. Toth, A.M. Schmidt, U.I. Tuor, G. Francis, T. Foniok, V. Brussee, J. Kaur, S.F. Yan, J.A. Martinez, P.A. Barber, A. Buchan, D.W. Zochodne, Diabetes, leukoencephalopathy and rage, Neurobiol. Dis. 23 (2006) 445–461.
[18] S.C. Correia, R.X. Santos, C. Carvalho, S. Cardoso, E. Candeias, M.S. Santos, C.R. Oliveira, P.I. Moreira, Insulin signaling, glucose metabolism and mitochondria:
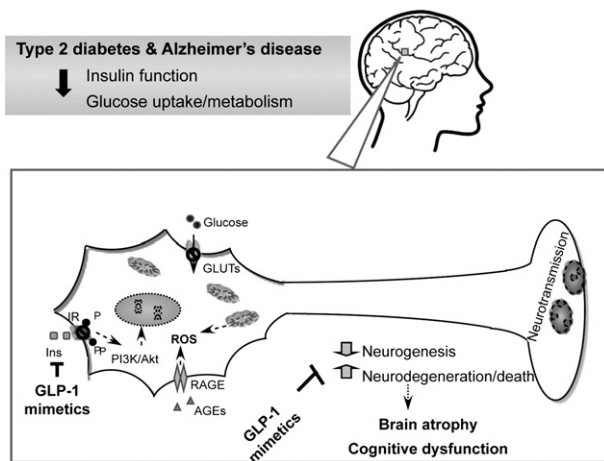
major players in Alzheimer's disease and diabetes interrelation, Brain Res. 1441 (2012) 64–78.

[19] J.S. Roriz-Filho, T.M. Sa-Roriz, I. Rosset, A.L. Camozzato, A.C. Santos, M.L. Chaves, J.C. Moriguti, M. Roriz-Cruz, (Pre)diabetes, brain aging, and cognition, Biochim. Biophys. Acta 1792 (2009) 432–443.

[20] H. Bruehl, O.T. Wolf, V. Sweat, A. Tirsi, S. Richardson, A. Convit, Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus, Brain Res. 1280 (2009) 186–194.

[21] M. Salkovic-Petrisic, S. Hoyer, Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach, J. Neural Transm. 72 (2007) 217–233.

[22] S. Craft, Insulin resistance syndrome and Alzheimer's disease: age- and obesity-related effects on memory, amyloid, and inflammation, Neurobiol. Aging 26 (Suppl.) (2005) S65–S69.

[23] Z. Laron, Insulin and the brain, Arch. Physiol. Biochem. 115 (2009) 112–116.

[24] A. Erol, An integrated and unifying hypothesis for the metabolic basis of sporadic Alzheimer's disease, J. Alzheimers Dis. 13 (2008) 241–253.

[25] R. Schechter, J. Whitmire, L. Holtzclaw, M. George, R. Harlow, S.U. Devaskar, Developmental regulation of insulin in the mammalian central nervous system, Brain Res. 582 (1992) 27–37.

[26] D. LeRoith, CNS regulation of carbohydrate metabolism, in: A. Szabo (Ed.), Advances in Metabolic Disorders, vol. 10, Academic Press, 1983, pp. 304–340.

[27] R.J. Schulingkamp, T.C. Pagano, D. Hung, R.B. Raffa, Insulin receptors and insulin action in the brain: review and clinical implications, Neurosci. Biobehav. Rev. 24 (2000) 855–872.

[28] G.S. Watson, E.R. Peskind, S. Asthana, K. Purganan, C. Wait, D. Chapman, M.W. Schwartz, S. Plymate, S. Craft, Insulin increases CSF Abeta42 levels in normal older adults, Neurology 60 (2003) 1899–1903.

[29] S. Hoyer, Memory function and brain glucose metabolism, Pharmacopsychiatry 36 (2003) S62–S67.

[30] G.G. Piroli, C.A. Grillo, M.J. Charron, B.S. McEwen, L.P. Reagan, Biphasic effects of stress upon GLUT8 glucose transporter expression and trafficking in the diabetic rat hippocampus, Brain Res. 1006 (2004) 28–35.

[31] A.M. Brands, G.J. Biessels, E.H. de Haan, L.J. Kappelle, R.P. Kessels, The effects of type 1 diabetes on cognitive performance: a meta-analysis, Diabetes Care 28 (2005) 726–735.

[32] S. Freude, L. Plum, J. Schnitker, U. Leeser, M. Udelhoven, W. Krone, J.C. Bruning, M. Schubert, Peripheral hyperinsulinemia promotes tau phosphorylation in vivo, Diabetes 54 (2005) 3343–3348.

[33] G.J. Biessels, A. Kamal, I.J. Urban, B.M. Spruijt, D.W. Erkelens, W.H. Gispen, Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment, Brain Res. 800 (1998) 125–135.

[34] Z.G. Li, W. Zhang, G. Grunberger, A.A. Sima, Hippocampal neuronal apoptosis in type 1 diabetes, Brain Res. 946 (2002) 221–231.

[35] A.A. Sima, Z.G. Li, The effect of C-peptide on cognitive dysfunction and hippocampal apoptosis in type 1 diabetic rats, Diabetes 54 (2005) 1497–1505.

[36] Z.G. Li, W. Zhang, A.A. Sima, Alzheimer-like changes in rat models of spontaneous diabetes, Diabetes 56 (2007) 1817–1824.

[37] R. Sankar, S. Thamotharan, D. Shin, K.H. Moley, S.U. Devaskar, Insulin-responsive glucose transporters—GLUT8 and GLUT4 are expressed in the developing mammalian brain, Mol. Brain Res. 107 (2002) 157–165.

[38] D. Porte, R.J. Seeley, S.C. Woods, D.G. Baskin, D.P. Figlewicz, M.W. Schwartz, Obesity, diabetes and the central nervous system, Diabetologia 41 (1998) 863–881.

[39] D.W. Clarke, L. Mudd, F.T. Boyd Jr., Insulin is released from rat brain neuronal cells in culture, J. Neurochem. 47 (1986) 831–836.

[40] J. Havrankova, J. Roth, M. Brownstein, Insulin receptors are widely distributed in the central nervous system of the rat, Nature 272 (1978) 827–829.

[41] B.E. Levin, Factors promoting and ameliorating the development of obesity, Physiol. Behav. 86 (2005) 633–639.

[42] J.L. Sievenpiper, A.L. Jenkins, D.L. Whitham, V. Vuksan, Insulin resistance: concepts, controversies, and the role of nutrition, Can. J. Diet. Pract. Res. 63 (2002) 20–32.

[43] Metabolic sensing neurons and the control of energy homeostasis, Physiol. Behav. 89 (2006) 486–489.

[44] D. Porte, D.G. Baskin, M.W. Schwartz, Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans, Diabetes 54 (2005) 1264–1276.

[45] B.E. Levin, V.H. Routh, L. Kang, N.M. Sanders, A.A. Dunn-Meynell, Neuronal glucosensing: what do we know after 50 years? Diabetes 53 (2004) 2521–2528.

[46] A. Wada, H. Yokoo, T. Yanagita, H. Kobayashi, New twist on neuronal insulin receptor signaling in health, disease, and therapeutics, J. Pharmacol. Sci. 99 (2005) 128–143.

[47] L. Plum, B.F. Belgardt, J.C. Brüning, Central insulin action in energy and glucose homeostasis, J. Clin. Invest. 116 (2006) 1761–1766.

[48] A. Pocai, T.K. Lam, R. Gutierrez-Juarez, S. Obici, G.J. Schwartz, J. Bryan, L. Aguilar-Bryan, L. Rossetti, Hypothalamic K(ATP) channels control hepatic glucose production, Nature 434 (2005) 1026–1031.

[49] J. Girard, The inhibitory effects of insulin on hepatic glucose production are both direct and indirect, Diabetes 55 (2006) S65–S69.

[50] W.H. Gispen, G.J. Biessels, Cognition and synaptic plasticity in diabetes mellitus, Trends Neurosci. 23 (2000) 542–549.

[51] J.B.C. Carvalheira, E.B. Ribeiro, E.P. Araújo, R.B. Guimarães, M.M. Telles, M. Torsoni, J.A. Gontijo, L.A. Velloso, M.J. Saad, Selective impairment of insulin signalling in the hypothalamus of obese Zucker rats, Diabetologia 46 (2003) 1629–1640.

[52] S. Boura-Halfon, Y. Zick, Phosphorylation of IRS proteins, insulin action, and insulin resistance, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E581–E591.

[53] G.J. Morton, D.E. Cummings, D.G. Baskin, G.S. Barsh, M.W. Schwartz, Central nervous system control of food intake and body weight, Nature 443 (2006) 289–295.

[54] A.L. McCall, Cerebral glucose metabolism in diabetes mellitus, Eur. J. Pharmacol. 490 (2004) 147–158.

[55] M.W. Schwartz, D.P. Figlewicz, D.G. Baskin, S.C. Woods, D. Porte, Insulin in the brain: a hormonal regulator of energy balance, Endocr. Rev. 13 (1992) 387–414.

[56] K.A. Heidenreich, D. Brandenburg, Oligosaccharide heterogeneity of insulin receptors. Comparison of N-linked glycosylation of insulin receptors in adipocytes and brain, Endocrinology 118 (1986) 1835–1842.

[57] D.W. Clarke, F.T. Boyd, M.S. Kappy, K. Raizada, Insulin binds to specific receptors and stimulates 2-deoxy-D-glucose uptake in cultured glial cells from rat brain, J. Biol. Chem. 259 (1984) 11672–11675.

[58] C.R. Park, Cognitive effects of insulin in the central nervous system, Neurosci. Biobehav. Rev. 25 (2001) 311–323.

[59] T.J. Huang, A. Verkhratsky, P. Fernyhough, Insulin enhances mitochondrial inner membrane potential and increases ATP levels through phosphoinositide 3-kinase in adult sensory neurons, Mol. Cell. Neurosci. 28 (2005) 42–54.

[60] S. Hoyer, L. Frölich, Brain function and insulin signal transduction in sporadic Alzheimer's disease, in: M. Sich (Ed.), Research Progress in Alzheimer's Disease and Dementia, Nova Science, New York, NY, USA, 2006, pp. 23–46.

[61] W. Zhao, H. Chen, H. Xu, E. Moore, N. Meiri, M.J. Quon, D.L. Alkon, Brain insulin receptors and spatial memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats, J. Biol. Chem. 274 (1999) 34893–34902.

[62] S.J. Vannucci, E.M. Koehler-Stec, K. Li, T.H. Reynolds, R. Clark, I.A. Simpson, GLUT4 glucose transporter expression in rodent brain: effect of diabetes, Brain Res. 797 (1998) 1–11.

[63] S. Craft, G.S. Watson, Insulin and neurodegenerative disease: shared and specific mechanisms, Lancet Neurol. 3 (2004) 169–178.

[64] E.M. Bingham, D. Hopkins, D. Smith, A. Pernet, W. Hallett, L. Reed, P.K. Marsden, S.A. Amiel, The role of insulin in human brain glucose metabolism: an 18fluorodeoxyglucose positron emission tomography study, Diabetes 51 (2002) 3384–3390.

[65] A. Ghosh, Y.C. Yuk, B.C. Mansfield, J.Y. Chou, Brain contains a functional glucose-6-phosphatase complex capable of endogenous glucose production, J. Biol. Chem. 280 (2005) 11114–11119.

[66] J.W. Russell, D. Golovoy, A.M. Vincent, P. Mahendru, J.A. Olzmann, A. Mentzer, E.L. Feldman, High glucose induced oxidative stress and mitochondrial dysfunction in neurons, FASEB J. 16 (2002) 1738–1748.

[67] A.I. Duarte, T. Proença, C.R. Oliveira, M.S. Santos, A.C. Rego, Insulin restores metabolic function in cultured cortical neurons subjected to oxidative stress, Diabetes 55 (2006) 2863–2870.

[68] A.I. Duarte, P. Santos, C.R. Oliveira, M.S. Santos, A.C. Rego, Insulin neuroprotection against oxidative stress is mediated by Akt and GSK-3β signaling pathways and changes in protein expression, Biochim. Biophys. Acta 1783 (2008) 994–1002.

[69] J.R. Koo, N.D. Vaziri, Effects of diabetes, insulin and antioxidants on NO synthase abundance and NO interaction with reactive oxygen species, Kidney Int. 63 (2003) 195–201.

[70] S.G. Hasselbalch, G.M. Knudsen, C. Videbaek, L.H. Pinborg, J.F. Schmidt, S. Holm, O.B. Paulson, No effect of insulin on glucose blood–brain barrier transport and cerebral metabolism in humans, Diabetes 48 (1999) 1915–1921.

[71] E.R. Seaquist, G.S. Damberg, I. Tkac, R. Gruetter, The effect of insulin on in vivo cerebral glucose concentrations and rates of glucose transport/metabolism in humans, Diabetes 50 (2001) 2203–2209.

[72] C. Benedict, M. Hallschmid, A. Hatke, B. Schultes, H.L. Fehm, J. Born, W. Kern, Intranasal insulin improves memory in humans, Psychoneuroendocrinology 29 (2004) 1326–1334.

[73] W. Kern, A. Peters, B. Fruehwald-Schultes, E. Deininger, J. Born, H.L. Fehm, Improving influence of insulin on cognitive functions in humans, Neuroendocrinology 74 (2001) 270–280.

[74] A.I. Duarte, M.S. Santos, R. Seiça, C.R. Oliveira, Oxidative stress affects synaptosomal γ-aminobutyric acid and glutamate transport in diabetic rats: the role of insulin, Diabetes 53 (2004) 2110–2116.

[75] A.I. Duarte, M.S. Santos, R. Seiça, C.R. Oliveira, Insulin affects synaptosomal GABA and glutamate transport under oxidative stress conditions, Brain Res. 977 (2003) 23–30.

[76] S. Craft, E. Peskind, M.W. Schwartz, G.D. Schellenberg, M. Raskind, D. Porte Jr., Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype, Neurology 50 (1998) 164–168.

[77] E. Steen, B.M. Terry, E.J. Rivera, J.L. Cannon, T.R. Neely, R. Tavares, X.J. Xu, J.R. Wands, S.M. de la Monte, Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J. Alzheimers Dis. 7 (2005) 63–80.

[78] S.M. de la Monte, J.R. Wands, Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease, J. Alzheimers Dis. 7 (2005) 45–61.

[79] L. Gasparini, H. Xu, Potential roles of insulin and IGF-1 in Alzheimer's disease, Trends Neurosci. 26 (2003) 404–406.

[80] W.Q. Qiu, M.F. Folstein, Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis, Neurobiol. Aging 27 (2006) 190–198.

[81] L. Ho, W. Qin, P.N. Pompl, Z. Xiang, J. Wang, Z. Zhao, Y. Peng, G. Cambareri, A. Rocher, C.V. Mobbs, P.R. Hof, G.M. Pasinetti, Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease, FASEB J. 18 (2004) 902–904.

[82] H. Fakhrai-Rad, A. Nikoshkov, A. Kamel, M. Fernstrom, J.R. Zierath, S. Norgren, H. Luthman, J. Galli, Insulin-degrading enzyme identified as a candidate diabetes susceptibility gene in GK rats, Hum. Mol. Genet. 9 (2000) 2149–2158.

[83] M.C. Leal, A. Fernandez-Gamba, L. Morelli, E.M. Castano, Cerebral proteolysis of amiloid-b peptide: relevance of insulin-degrading enzyme in Alzheimer's disease, Medicina (B Aires) 69 (2009) 466–472.

[84] H.W. Querfurth, F.M. LaFerla, Alzheimer's disease, N. Engl. J. Med. 362 (2010) 329–344.

[85] L. Gasparini, G.K. Gouras, R. Wang, R.S. Gross, M.F. Beal, P. Greengard, H. Xu, Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signalling, J. Neurosci. 21 (2001) 2561–2570.

[86] C.J. Phiel, C.A. Wilson, V.M. Lee, P.S. Klein, GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides, Nature 423 (2003) 435–439.

[87] Z. Kroner, The relationship between Alzheimer's disease and diabetes: type 3 diabetes? Altern. Med. Rev. 14 (2009) 373–379.

[88] K. Talbot, H.Y. Wang, H. Kazi, L.Y. Han, K.P. Bakshi, A. Stucky, R.L. Fuino, K.R. Kawaguchi, A.J. Samoyedny, R.S. Wilson, Z. Arvanitakis, J.A. Schneider, B.A. Wolf, D.A. Bennett, J.Q. Trojanowski, S.E. Arnold, Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline, J. Clin. Invest. 122 (2012) 1316–1338.

[89] N. Lester-Coll, E.J. Rivera, S.J. Soscia, K. Doiron, J.R. Wands, S.M. de la Monte, Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease, J. Alzheimers Dis. 9 (2006) 13–33.

[90] C.M. Cheng, V. Tseng, J. Wang, D. Wang, L. Matyakhina, C.A. Bondy, Tau is hyperphosphorylated in the insulin-like growth factor-I null brain, Endocrinology 146 (2005) 5086–5091.

[91] M. Schubert, D.P. Brazil, D.J. Burks, J.A. Kushner, J. Ye, C.L. Flint, J. Farhang-Fallah, P. Dikkes, X.M. Warot, C. Rio, G. Corfas, M.F. White, Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation, J. Neurosci. 23 (2003) 7084–7092.

[92] M. Schubert, D. Gautam, D. Surjo, K. Ueki, S. Baudler, D. Schubert, T. Kondo, J. Alber, N. Galldiks, E. Kustermann, S. Arndt, A.H. Jacobs, W. Krone, C.R. Kahn, J.C. Bruning, Role for neuronal insulin resistance in neurodegenerative diseases, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 3100–3105.

[93] C.G. Jolivalt, C.A. Lee, K.K. Beiswenger, J.L. Smith, M. Orlov, M.A. Torrance, E. Masliah, Defective insulin signalling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: parallels with Alzheimer's disease and correction by insulin, J. Neurosci. Res. 86 (2008) 3265–3274.

[94] C.G. Jolivalt, R. Hurford, C.A. Lee, W. Dumaop, E. Rockenstein, E. Masliah, Type 1 diabetes exaggerates features of Alzheimer's disease in APP transgenic mice, Exp. Neurol. 223 (2010) 422–431.

[95] M. Lesort, G.V. Johnson, Insulin-like growth factor-1 and insulin mediate transient site-selective increases in tau phosphorylation in primary cortical neurons, Neuroscience 99 (2000) 305–316.

[96] B. Kim, C. Backus, S. Oh, J.M. Hayes, E.L. Feldman, Increased tau phosphorylation and cleavage in mouse models of type 1 and type 2 diabetes, Endocrinology 150 (2009) 5294–5301.

[97] W.Q. Zhao, D.L. Alkon, Role of insulin and insulin receptor in learning and memory, Mol. Cell. Endocrinol. 177 (2001) 125–134.

[98] L. Gasparini, W.J. Netzer, P. Greengard, H. Xu, Does insulin dysfunction play a role in Alzheimer's disease? Trends Pharmacol. Sci. 23 (2002) 288–293.

[99] V. Brussee, F.A. Cunningham, D.W. Zochodne, Direct insulin signalling of neurons reverses diabetic neuropathy, Diabetes 53 (2004) 1824–1830.

[100] Y. Soeda, H. Tsuneki, H. Muranaka, N. Mori, S. Hosoh, Y. Ichihara, S. Kagawa, X. Wang, N. Toyooka, Y. Takamura, T. Uwano, H. Nishijo, T. Wada, T. Sasaoka, The inositol phosphatase SHIP2 negatively regulates insulin/IGF-I actions implicated in neuroprotection and memory function in mouse brain, Mol. Endocrinol. 24 (2010) 1965–1977.

[101] L.P. van der Heide, G.M.J. Ramakers, M.P. Smidt, Insulin signaling in the central nervous system: learning to survive, Prog. Neurobiol. 79 (2006) 205–221.

[102] M. Ristow, Neurodegenetive disorders associated with diabetes mellitus, J. Mol. Med. 82 (2004) 510–529.

[103] G.S. Watson, S. Craft, Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease, Eur. J. Pharmacol. 490 (2004) 97–113.

[104] O. Kann, R. Kovacs, Mitochondria and neuronal activity, Am. J. Physiol. Cell Physiol. 292 (2007) C641–C657.

[105] A. Scheepers, H.G. Joost, A. Schurmann, The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function, J. Parenter. Enter. Nutr. 28 (2004) 364–371.

[106] W.Q. Zhao, M. Townsend, Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease, Biochim. Biophys. Acta 1792 (2009) 482–496.

[107] L.D. Baker, D.J. Cross, S. Minoshima, D. Belongia, G.S. Watson, S. Craft, Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes, Arch. Neurol. 68 (2011) 51–57.

[108] F. Chiti, C.M. Dobson, Protein misfolding, functional amyloid, and human disease, Annu. Rev. Biochem. 75 (2006) 333–366.

[109] P.I. Moreira, Alzheimer's disease and diabetes: an integrative view of the role of mitochondria, oxidative stress, and insulin, J. Alzheimers Dis. 30 (2012) S199–S215.

[110] A.M. Schmeichel, J.D. Schmelzer, P.A. Low, Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy, Diabetes 52 (2003) 165–171.

[111] G.M. Leinninger, C. Backus, A.M. Sastry, Y.B. Yi, C.W. Wang, E.L. Feldman, Mitochondria in DRG neurons undergo hyperglycemic mediated injury through Bim, Bax and the fission protein Drp1, Neurobiol. Dis. 23 (2006) 11–22.

[112] J.L. Edwards, A. Quattrini, S.I. Lentz, C. Figueroa-Romero, F. Cerri, C. Backus, Y. Hong, E.L. Feldman, Diabetes regulates mitochondrial biogenesis and fission in mouse neurons, Diabetologia 53 (2010) 160–169.

[113] C. Sims-Robinson, B. Kim, A. Rosko, E.L. Feldman, How does diabetes accelerate Alzheimer disease pathology? Nat. Rev. Neurol. 6 (2010) 551–559.

[114] P.I. Moreira, M.S. Santos, A.M. Moreno, R. Seiça, C.R. Oliveira, Increased vulnerability of brain mitochondria in diabetic (Goto-Kakizaki) rats with aging and amyloid-β exposure, Diabetes 52 (2003) 1449–1456.

[115] S.M. Cardoso, M.T. Proença, S. Santos, I. Santana, C.R. Oliveira, Cytochrome c oxidase is decreased in Alzheimer's disease platelets, Neurobiol. Aging 25 (2004) 105–110.

[116] N. Dragicevic, M. Mamcarz, Y. Zhu, R. Buzzeo, J. Tan, G.W. Arendash, P.C. Bradshaw, Mitochondrial amyloid-beta levels are associated with the extent of mitochondrial dysfunction in different brain regions and the degree of cognitive impairment in Alzheimer's transgenic mice, J. Alzheimers Dis. 20 (Suppl. 2) (2010) S535–S550.

[117] P.I. Moreira, A.P. Rolo, C. Sena, R. Seica, C.R. Oliveira, M.S. Santos, Insulin attenuates diabetes-related mitochondrial alterations: a comparative study, Med. Chem. 2 (2006) 299–308.

[118] R. Mastrocola, F. Restivo, I. Vercellinatto, O. Danni, E. Brignardello, M. Aragno, G. Boccuzzi, Oxidative and nitrosative stress in brain mitochondria of diabetic rats, J. Endocrinol. 187 (2005) 37–44.

[119] D.A. Butterfield, M. Perluigi, R. Sultana, Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics, Eur. J. Pharmacol. 545 (2006) 39–50.

[120] J.P. Silva, M. Kohler, C. Graff, A. Oldfors, M.A. Magnuson, P.O. Berggren, N.G. Larsson, Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes, Nat. Genet. 26 (2000) 336–340.

[121] P.I. Moreira, M.S. Santos, C. Sena, E. Nunes, R. Seiça, C.R. Oliveira, CoQ10 therapy attenuates amyloid β-peptide toxicity in brain mitochondria isolated from aged diabetic rats, Exp. Neurol. 196 (2005) 112–119.

[122] P.I. Moreira, M.S. Santos, C. Sena, R. Seiça, C.R. Oliveira, Insulin protects against amyloid β-peptide toxicity in brain mitochondria of diabetic rats, Neurobiol. Dis. 18 (2005) 628–637.

[123] S. Correia, C. Carvalho, M.S. Santos, T. Proença, E. Nunes, A.I. Duarte, P. Monteiro, R. Seiça, C.R. Oliveira, P.I. Moreira, Metformin protects the brain against the oxidative imbalance promoted by type 2 diabetes, Med. Chem. 4 (2008) 358–364.

[124] M.S. Santos, A.I. Duarte, M.J. Matos, T. Proença, R. Seiça, C.R. Oliveira, Synaptosomes isolated from Goto-Kakizaki diabetic rat brain exhibit increased resistance to oxidative stress: role of vitamin E, Life Sci. 67 (2000) 3061–3073.

[125] P. Fernyhough, S.K. Roy Chowdhury, R.E. Schmidt, Mitochondrial stress and the pathogenesis of diabetic neuropathy, Expert. Rev. Endocrinol. Metab. 5 (2010) 39–49.

[126] V. Frisardi, V. Solfrizzi, D. Seripa, C. Capurso, A. Santamato, D. Sancarlo, G. Vendemiale, A. Pilotto, F. Panza, Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease, Ageing Res. Rev. 9 (2010) 399–417.

[127] J. Vasquez-Vivar, B. Kalyanaraman, M.C. Kennedy, Mitochondrial aconitase is a source of hydroxyl radical. An electron spin resonance investigation, J. Biol. Chem. 275 (2000) 14064–14069.

[128] M. Patel, B.J. Day, J.D. Crapo, I. Fridovich, J.O. McNamara, Requirement for superoxide in excitotoxic cell death, Neuron 16 (1996) 345–355.

[129] H. Liu, H. Wang, S. Shenvi, T.M. Hagen, R.M. Liu, Glutathione metabolism during aging and in Alzheimer disease, Ann. N. Y. Acad. Sci. 1019 (2004) 346–349.

[130] R. Resende, P.I. Moreira, T. Proença, A. Deshpande, J. Busciglio, C. Pereira, C.R. Oliveira, Brain oxidative stress in a tripletransgenic mouse model of Alzheimer disease, Free Radic. Biol. Med. 44 (2008) 2051–2057.

[131] T. Peerapatdit, N. Patchanans, A. Likidlilid, S. Poldee, C. Sriratanasathavorn, Plasma lipid peroxidation and antioxidiant nutrients in type 2 diabetic patients, J. Med. Assoc. Thai. 89 (Suppl. 5) (2006) S147–S155.

[132] I. Baldeiras, I. Santana, M.T. Proença, M.H. Garrucho, R. Pascoal, A. Rodrigues, D. Duro, C.R. Oliveira, Peripheral oxidative damage in mild cognitive impairment and mild Alzheimer's disease, J. Alzheimers Dis. 15 (2008) 117–128.

[133] M. Manczak, T.S. Anekonda, E. Henson, B.S. Park, J. Quinn, P.H. Reddy, Mitochondria are a direct site of A beta accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression, Hum. Mol. Genet. 15 (2006) 1437–1449.

[134] A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Mitochondrial permeability transition and oxidative stress, FEBS Lett. 495 (2001) 12–15.

[135] A.M. Vincent, L.L. McLean, C. Backus, E.L. Feldman, Short-term hyperglycemia produces oxidative damage and apoptosis in neurons, FASEB J. 19 (2005) 638–640.

[136] Z.G. Li, W. Zhang, A.A.F. Sima, The role of impaired insulin/IGF action in primary diabetic encephalopathy, Brain Res. 1037 (2005) 12–24.

[137] S. Correia, C. Carvalho, M.S. Santos, R. Seiça, C.R. Oliveira, P.I. Moreira, Mechanisms of action of metformin in type 2 diabetes and associated complications: an overview, Mini-Rev. Med. Chem. 8 (2008) 1343–1354.

[138] I.G. Obrosova, V.R. Drel, P. Pacher, O. Ilnytska, Z.Q. Wang, M.J. Stevens, M.A. Yorek, Oxidative nitrosative stress and poly(ADP-ribose) polymerase (PARP) activation in experimental diabetic neuropathy: the relation is revisited, Diabetes 54 (2005) 3435–3441.

[139] S.Y. Goh, M.E. Cooper, Clinical review: the role of advanced glycation end products in progression and complications of Diabetes, J. Clin. Endocrinol. Metab. 93 (2008) 1143–1152.

[140] C. Carvalho, S.C. Correia, R.X. Santos, S. Cardoso, P.I. Moreira, T.A. Clark, X. Zhu, M.A. Smith, G. Perry, Role of mitochondrial-mediated signaling pathways in Alzheimer disease and hypoxia, J. Bioenerg. Biomembr. 41 (2009) 433–440.

[141] X. Girones, A. Guimera, C.Z. Cruz-Sanchez, A. Ortega, N. Sasaki, Z. Makita, J.V. Lafuente, R. Kalaria, F.F. Cruz-Sanchez, N- epsilon-carboxymethyllysine in brain aging, diabetes mellitus, and Alzheimer's disease, Free Radic. Biol. Med. 36 (2004) 1241–1247.

[142] A. Gasser, J.M. Forbes, Advanced glycation: implications in tissue damage and disease, Protein Pept. Lett. 15 (2008) 385–391.

[143] B. Engelsen, F. Fonnum, Effects of hypoglycemia on the transmitter pool and the metabolic pool of glutamate in rat brain, Neurosci. Lett. 42 (1983) 317–322.

[144] M. Erecinska, M.M. Zaleska, I. Nissim, D. Nelson, F. Dagani, M. Yudkoff, Glucose and synaptosomal glutamate metabolism: studies with [^{15}N]glutamate, J. Neurochem. 51 (1988) 892–902.

[145] H.S. Waagepetersen, I.J. Bakken, O.M. Larsson, U. Sonnewald, A. Schousboe, Comparison of lactate and glucose metabolism in cultured neocortical neurons and astrocytes using 13C-NMR spectroscopy, Dev. Neurosci. 20 (1998) 310–320.

[146] S. Cardoso, C. Carvalho, R. Santos, S. Correia, M.S. Santos, R. Seiça, C.R. Oliveira, P.I. Moreira, Impact of STZ-induced hyperglycemia and insulin-induced hypoglycemia in plasma amino acids and cortical synaptosomal neurotransmitters, Synapse 65 (2011) 457–466.

[147] L.K. Bak, A. Schousboe, H.S. Waagepetersen, The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer, J. Neurochem. 98 (2006) 641–653.

[148] F. Frigerio, M. Casimir, S. Carobbio, P. Maechler, Tissue specificity of mitochondrial glutamate pathways and the control of metabolic homeostasis, Biochim. Biophys. Acta 1777 (2008) 965–972.

[149] B.A. Menge, H. Schrader, P.R. Ritter, M. Ellrichmann, W. Uhl, W.E. Schmidt, J.J. Meier, Selective amino acid deficiency in patients with impaired glucose tolerance and type 2 diabetes, Regul. Pept. 160 (2010) 75–80.

[150] L.L. Guyot, F.G. Diaz, M.H. O'Regan, D. Song, J.W. Phillis, The effect of topical insulin on the release of excitotoxic and other amino acids from the rat cerebral cortex during streptozotocin-induced hyperglycemic ischemia, Brain Res. 872 (2000) 29–36.

[151] J.W. Phillis, D. Song, M.H. O'Regan, Effect of hyperglycemia on extracellular levels of amino acids and free fatty acids in the ischemic/reperfused rat cerebral cortex, Brain Res. 837 (1999) 177–183.

[152] L.L. Guyot, F.G. Diaz, M.H. O'Regan, D. Song, J.W. Phillis, Topical insulin and accumulation of excitotoxic and other amino acids in ischemic rat cerebral cortex, Exp. Biol. Med. 224 (2000) 28–31.

[153] G.J. Biessels, L.P. Van der Heide, A. Kamal, R.L.A.W. Bleys, W.H. Gispen, Ageing and diabetes: implications for brain function, Eur. J. Pharmacol. 441 (2002) 1–14.

[154] C. Vilchis, R. Salceda, Effect of diabetes on levels and uptake of putative amino acid neurotransmitters in rat retina and retinal pigment epithelium, Neurochem. Res. 21 (1996) 1167–1171.

[155] J. Beauquis, F. Saravia, J. Coulaud, P. Roig, M. Dardenne, F. Homo-Delarche, A. De Nicola, Prominently decreased hippocampal neurogenesis in a spontaneous model of type 1 diabetes, the nonobese diabetic mouse, Exp. Neurol. 210 (2008) 359–367.

[156] A.M. Stranahan, T.V. Arumugam, R.G. Cutler, K. Lee, J.M. Egan, M.P. Mattson, Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons, Nat. Neurosci. 11 (2008) 309–317.

[157] W.J. Zhang, Y.F. Tan, J.T. Yue, M. Vranic, J.M. Wojtowicz, Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats, Acta Neurol. Scand. 117 (2008) 205–210.

[158] B.T. Lang, Y. Yan, R.J. Dempsey, R. Vemuganti, Impaired neurogenesis in adult type-2 diabetic rats, Brain Res. 3 (2009) 25–33.

[159] A. Lindqvist, P. Mohapel, B. Bouter, H. Frielingsdorf, D. Pizzo, P. Brundin, C. Erlanson-Albertsson, High-fat diet impairs hippocampal neurogenesis in male rats, Eur. J. Neurol. 13 (2006) 1385–1388.

[160] A. Hamilton, S. Patterson, D. Porter, V.A. Gault, C. Holscher, Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain, J. Neurosci. Res. 89 (2011) 481–489.

[161] C. Wiltrout, B. Lang, Y. Yan, R.J. Dempsey, R. Vemuganti, Repairing brain after stroke: a review on post-ischemic neurogenesis, Neurochem. Int. 50 (2007) 1028–1041.

[162] S. Das, A. Basu, Inflammation: a new candidate in modulating adult neurogenesis, J. Neurosci. Res. 86 (2008) 1199–1208.

[163] C.T. Ekdahl, Z. Kokaia, O. Lindvall, Brain inflammation and adult neurogenesis: the dual role of microglia, Neuroscience (2008), http://dx.doi.org/10.1016/j.neuroscience.2008.06.052.

[164] A.M.J. MacLullich, J.R. Seckl, Diabetes and cognitive decline: are steroids the missing link? Cell Metab. 7 (2008) 286–287.

[165] C. Messier, Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging, Neurobiol. Aging 26 (2005) S26–S30.

[166] M.S. Oitzl, M. Fluttert, W. Sutanto, E.R. de Kloet, Continuous blockade of brain glucocorticoid receptors facilitates spatial learning and memory in rats, Eur. J. Neurosci. 10 (1998) 3759–3766.

[167] C.T. Kodl, E.R. Seaquist, Cognitive dysfunction and diabetes mellitus, Endocr. Rev. 29 (2008) 494–511.

[168] N.N. Johnson-Farley, T. Travkina, D.S. Cowen, Cumulative activation of Akt and consequent inhibition of glycogen synthase kinase-3 by brain-derived neurotrophic factor and insulin-like growth factor-1 in cultured hippocampal neurons, J. Pharmacol. Exp. Ther. 316 (2006) 1062–1069.

[169] P. Singh, A. Jain, G. Kaur, Impact of hypoglycaemia and diabetes on CNS: correlation of mitochondrial oxidative stress with DNA damage, Mol. Cell. Biochem. 260 (2004) 153–159.

[170] R.C. Turner, C.A. Cull, V. Frighi, R.R. Holman, Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus. Progressive requirement for multiple therapies (UKPDS 49), J. Am. Med. Assoc. 281 (1999) 2005–2012.

[171] R.N. Auer, Hypoglycemic brain damage, Metab. Brain Dis. 19 (2004) 169–175.

[172] S.W. Suh, A.M. Hamby, R.A. Swanson, Hypoglycemia, brain energetics, and hypoglycemic neuronal death, Glia 55 (2007) 1280–1286.

[173] S. Cardoso, M.S. Santos, R. Seiça, P.I. Moreira, Cortical and hippocampal mitochondria bioenergetics and oxidative status during hyperglycemia and/or insulin-induced hypoglycemia, Biochim. Biophys. Acta 1802 (2010) 942–951.

[174] J.E. McGowan, S.A. Zanelli, A.G. Haynes-Laing, O.P. Mishra, M. Delivoria-Papadopoulos, Modification of glutamate binding sites in newborn brain during hypoglycemia, Brain Res. 927 (2002) 80–86.

[175] A. Battezzati, S. Benedini, A. Fattorini, L. Piceni Sereni, L. Luzi, Effect of hypoglycemia on amino acid and protein metabolism in healthy humans, Diabetes 49 (2000) 1543–1551.

[176] A. Battezzati, S. Benedini, L.P. Sereni, F. DeTaddeo, P. Maffi, A. Secchi, L. Luzi, Protein and glutamine kinetics during counter-regulatory failure in type 1 diabetes, Nutr. Metab. Cardiovasc. Dis. 7 (2009) 352–357.

[177] C.M. Palmeira, M.S. Santos, A.P. Carvalho, C.R. Oliveira, Membrane lipid peroxidation induces changes in aminobutyric acid transport and calcium uptake by synaptosomes, Brain Res. 609 (1993) 117–123.

[178] R. Sah, R.D. Schwartz-Bloom, Optical imaging reveals elevated intracellular chloride in hippocampal pyramidal neurons after oxidative stress, J. Neurosci. 19 (1999) 9209–9217.

[179] P. Saransaari, S.S. Oja, Alanine release from the adult and developing hippocampus is enhanced by ionotropic glutamate receptor agonists and cell-damaging conditions, Neurochem. Res. 24 (1999) 407–414.

[180] S. Chen, A.R. Liu, F.M. An, W.B. Yao, X.D. Gao, Amelioration of neurodegenerative changes in cellular and rat models of diabetes-related Alzheimer's disease byexendin-4, Age (Dordr) 34 (2012) 1211–1224, (PMID: 21901364).

[181] G.C. Alexander, N.L. Sehgal, R.M. Moloney, R.S. Stafford, National trends in treatment of type 2 diabetes mellitus, 1994–2007, Arch. Intern. Med. 168 (2008) 2088–2094.

[182] D.M. Nathan, J.B. Buse, M.B. Davidson, E. Ferrannini, R.R. Holman, R. Sherwin, B. Zinman, for the American Diabetes Association; European Association for the Study of Diabetes, Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes, Diabetes Care 32 (2009) 193–203.

[183] H.W. Rodbard, P.S. Jellinger, J.A. Davidson, D. Einhorn, A.J. Garber, G. Grunberger, Y. Handelsman, E.S. Horton, H. Lebovitz, P. Levy, E.S. Moghissi, S.S. Schwartz, AACE/ACE consensus statement: statement by an American Association of Clinical Endocrinologists/American College of Endocrinology Consensus Panel on type 2 diabetes mellitus: an algorithm for glycemic control, Endocr. Pract. 15 (2009) 540–559.

[184] A. Cimini, M.P. Cerù, Emerging roles of peroxisome proliferator-activated receptors (PPARs) in the regulation of neural stem cells proliferation and differentiation, Stem Cell Rev. 4 (2008) 293–303.

[185] K. Tureyen, R. Kapadia, K.K. Bowen, I. Satriotomo, J. Liang, D.L. Feinstein, R. Vemuganti, Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type2 diabetic rodents, J. Neurochem. 101 (2007) 41–56.

[186] W.A. Pedersen, P.J. McMillan, J.J. Kulstad, J.B. Leverenz, S. Craft, G.R. Haynatzki, Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice, Exp. Neurol. 199 (2006) 265–273.

[187] J. Buse, Combining insulin and oral agents, Am. J. Med. 108 (2000) 23–32.

[188] W.A. Hsueh, L.M. Moore, M. Bryer-Ash, Pathophysiology of type 2 diabetes: a quartet of abnormalities, in: W.A. Hsueh, L.M. Moore, M. Bryer-Ash (Eds.), Contemporary Diagnosis and Management of Type 2 Diabetes, Handbooks in Health Care, Newtown, Pa, USA, 2004, pp. 5–19.

[189] R.K. Campbell, J.R. White, More choices than ever before: emerging therapies for type 2 diabetes, Diabetes Educ. 34 (2008) 518–534.

[190] H. Elrick, L. Stimmler, C.J. Hlad Jr., Y. Arai, Plasma insulin response to oral and intravenous glucose administration, J. Clin. Endocrinol. Metab. 24 (1964) 1076–1082.

[191] D.J. Drucker, M.A. Nauck, The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes, Lancet 368 (2006) 1696–1705.

[192] T. Vilsbøll, J.J. Holst, Incretins, insulin secretion and type 2 diabetes mellitus, Diabetologia 47 (2004) 357–366.

[193] M.B. Toft-Nielsen, M.B. Damholt, S. Madsbad, L.M. Hilsted, T.E. Hughes, B.K. Michelsen, J.J. Holst, Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients, J. Clin. Endocrinol. 86 (2001) 3717–3723.

[194] M.B. Toft-Nielsen, S. Madsbad, J.J. Holst, Determinants of the effectiveness of glucagon-like peptide-1 in type 2 diabetes, J. Clin. Endocrinol. 86 (2001) 3853–3860.

[195] R.E. Amori, J. Lau, A.G. Pittas, Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis, JAMA 298 (2007) 194–206.

[196] T.J. Kieffer, C.H. McIntosh, R.A. Pederson, Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase-IV, Endocrinology 136 (1995) 3585–3596.

[197] B. Ahrén, DPP-4 inhibitors, Best Pract. Res. Clin. Endocrinol. Metab. 21 (2007) 517–533.

[198] C. Morimoto, S.F. Schlossman, The structure and function of CD26 in the T-cell immune response, Immunol. Rev. 161 (1998) 55–70.

[199] M.A. Nauck, T. Vilsbøll, B. Gallwitz, A. Garber, S. Madsbad, Incretin-based therapies. Viewpoints on the way to consensus, Diabetes Care 32 (2009) S223–S231.

[200] D.J. Drucker, S.I. Sherman, F.S. Gorelick, R.M. Bergenstal, R.S. Sherwin, J.B. Buse, Incretin-based therapies for the treatment of type 2 diabetes: evaluation of the risks and benefits, Diabetes Care 33 (2010) 428–433.

[201] B.J. Lamont, D.J. Drucker, Differential antidiabetic efficacy of incretin agonists versus DPP-4 inhibition in high fat fed mice, Diabetes 57 (2008) 190–198.

[202] B. Gallwitz, Glucagon-like peptide-1 analogues for type 2 diabetes mellitus: current and emerging agents, Drugs 71 (2011) 1675–1688.
[203] J.A. Lovshin, D.J. Drucker, Incretin-based therapies for type 2 diabetes mellitus, Nat. Rev. Endocrinol. 5 (2009) 262–269.
[204] R.A. DeFronzo, T. Okerson, P. Viswanathan, X. Guan, J.H. Holcombe, L. MacConell, Effects of exenatide versus sitagliptin on postprandial glucose, insulin and glucagon secretion, gastric emptying, and caloric intake: a randomized, cross-over study, Curr. Med. Res. Opin. 24 (2008) 2943–2952.
[205] R.M. Bergenstal, C. Wysham, L. Macconell, J. Malloy, B. Walsh, P. Yan, K. Wilhelm, J. Malone, L.E. Porter, DURATION-2 Study Group, Efficacy and safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial, Lancet 376 (2010) 431–439.
[206] R.E. Pratley, M. Nauck, T. Bailey, E. Montanya, R. Cuddihy, S. Filetti, A.B. Thomsen, R.E. Søndergaard, M. Davies, 1860-LIRA-DPP-4 Study Group, Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycemic control with metformin: a 26-week, randomized, parallelgroup, open-label trial, Lancet 375 (2010) 1447–1456.
[207] E. Alvarez, M.D. Martínez, I. Roncero, J.A. Chowen, B. García-Cuartero, J.D. Gispert, C. Sanz, P. Vázquez, A. Maldonado, J. de Cáceres, M. Desco, M.A. Pozo, E. Blázquez, The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem, J. Neurochem. 92 (2005) 798–806.
[208] M. Körner, M. Stöckli, B. Waser, J.C. Reubi, GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting, J. Nucl. Med. 48 (2007) 736–743.
[209] A. Hamilton, C. Hölscher, Receptors for the incretin glucagon-like peptide-1 are expressed on neurons in the central nervous system, Neuroreport 20 (2009) 1161–1166.
[210] Y. Li, S. Chigurupati, H.W. Holloway, M. Mughal, D. Tweedie, D.A. Bruestle, M.P. Mattson, Y. Wang, B.K. Harvey, B. Ray, D.K. Lahiri, N.H. Greig, Exenatide ameliorates motor neuron degeneration in cellular and animal models of amyotrophic lateral sclerosis, PLoS One 7 (2012) e32008–E32021.
[211] J.J. Holst, T. Vilsbøll, C.F. Deacon, The incretin system and its role in type 2 diabetes mellitus, Mol. Cell. Endocrinol. 297 (2009) 127–136.
[212] C. Orskov, J. Jeppesen, S. Madsbad, J.J. Holst, Proglucagon products in plasma of non insulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine, J. Clin. Invest. 87 (1991) 415–423.
[213] M. Gutniak, C. Orskov, J.J. Holst, B. Ahrén, S. Efendic, Antidiabetogenic effect of glucagon-like peptide-1 (7–36) amide in normal subjects and patients with diabetes mellitus, N. Engl. J. Med. 336 (1992) 1316–1322.
[214] M. Zander, S. Madsbad, J.L. Madsen, J.J. Holst, Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study, Lancet 359 (2002) 824–830.
[215] E. Rask, T. Olsson, S. Söderberg, O. Johnson, J. Seckl, J.J. Holst, B. Ahrén, Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA), Impaired incretin response after a mixed meal is associated with insulin resistance in nondiabetic men, Diabetes Care 24 (2001) 1640–1645.
[216] E. Rask, T. Olsson, S. Söderberg, J.J. Holst, A. Tura, G. Pacini, B. Ahrén, Insulin secretion and incretin hormones after oral glucose in non-obese subjects with impaired glucose tolerance, Metabolism 53 (2004) 624–631.
[217] E. Muscelli, A. Mari, A. Casolaro, S. Camastra, G. Seghieri, A. Gastaldelli, J.J. Holst, E. Ferrannini, Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients, Diabetes 57 (2008) 1340–1348.
[218] S. Herzberg-Schäfer, M. Heni, N. Stefan, H.U. Häring, A. Fritsche, Impairment of GLP1-induced insulin secretion: role of genetic background, insulin resistance and hyperglycaemia, Diabetes Obes. Metab. 14 (2012) 85–90.
[219] E. Mannucci, A. Ognibene, F. Cremasco, G. Bardini, A. Mencucci, E. Pierazzuoli, S. Ciani, A. Fanelli, G. Messeri, C.M. Rotella, Glucagon-like peptide (GLP)-1 and leptin concentrations in obese patients with type 2 diabetes mellitus, Diabet. Med. 17 (2000) 713–719.
[220] F. Zhang, X. Tang, H. Cao, Q. Lü, N. Li, Y. Liu, X. Zhang, Y. Zhang, M. Cao, J. Wan, Z. An, N. Tong, Impaired secretion of total glucagon-like peptide 1 in people with impaired fastingglucose combined impaired glucose tolerance, Int. J. Med. Sci. 9 (2012) 574–581.
[221] T. Vilsbøll, T. Krarup, C.F. Deacon, S. Madsbad, J.J. Holst, Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients, Diabetes 50 (2001) 609–613.
[222] S. Lee, D. Yabe, K. Nohtomi, M. Takada, R. Morita, Y. Seino, T. Hirano, Intact glucagon-like peptide-1 levels are not decreased in Japanese patients with type 2 diabetes, Endocr. J. 7 (2010) 119–126.
[223] K. Vollmer, J.J. Holst, B. Baller, M. Ellrichmann, M.A. Nauck, W.E. Schmidt, J.J. Meier, Predictors of incretin concentrations in subjects with normal, impaired, and diabetic glucose tolerance, Diabetes 57 (2008) 678–687.
[224] A.M. Nauck, I. Vardarli, F.C. Deacon, J.J. Holst, J.J. Meier, Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? Diabetologia 54 (2011) 10–18.
[225] J. Kozawa, K. Okita, A. Imagawa, H. Iwahashi, J.J. Holst, K. Yamagata, I. Shimomura, Similar incretin secretion in obese and non-obese Japanese subjects with type 2 diabetes, Biochem. Biophys. Res. Commun. 393 (2010) 410–413.
[226] F. Rodriquez de Fonseca, M. Navarro, E. Alvarez, I. Roncero, J.A. Chowen, O. Maestre, R. Gómez, R.M. Muñoz, J. Eng, E. Blázquez, Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats, Metabolism 49 (2000) 709–717.
[227] B. Gedulin, K. Tatarkiewicz, P. Smith, J. Wilson, D. Bhole, D. Kendall, D. Hargrove, D. Parkes, Contrasting actions of sitagliptin and exenatide on food intake, body weight, glucose stimulated insulin secretion and gastric emptying in rodents (abstract no. 0576), Diabetologia 50 (Suppl. 1) (2007) S239.
[228] A.J. Kastin, V. Akerstrom, W. Pan, Interactions of glucagon-like peptide-1 (GLP-1) with the blood–brain barrier, J. Mol. Neurosci. 18 (2002) 7–14.
[229] M. Gil-Lozano, D. Pérez-Tilve, M. Alvarez-Crespo, A. Martís, A.M. Fernandez, P.A. Catalina, L.C. Gonzalez-Matias, F. Mallo, GLP-1(7–36)-amide and Exendin-4 stimulate the HPA axis in rodents and humans, Endocrinology 151 (2010) 2629–2640.
[230] D. Pérez-Tilve, L. González-Matías, M. Alvarez-Crespo, R. Leiras, S. Tovar, C. Diéguez, F. Mallo, Exendin-4 potently decreases ghrelin levels in fasting rats, Diabetes 56 (2007) 143–151.
[231] C. Knauf, P.D. Cani, D.H. Kim, M.A. Iglesias, C. Chabo, A. Waget, A. Colom, S. Rastrelli, N.M. Delzenne, D.J. Drucker, R.J. Seeley, R. Burcelin, Role of central nervous system glucagon-like peptide-1 receptors in enteric glucose sensing, Diabetes 57 (2008) 2603–2612.
[232] C. Hölscher, The role of GLP-1 in neuronal activity and neurodegeneration, Vitam. Horm. 84 (2010) 331–354.
[233] J.J. Holst, R. Burcelin, E. Nathanson, Neuroprotective properties of GLP-1: theoretical and practical applications, Curr. Med. Res. Opin. 27 (2011) 547–558.
[234] R. Isacson, E. Nielsen, K. Dannaeus, G. Bertilsson, C. Patrone, O. Zachrisson, L. Wikström, The glucagon-like peptide 1 receptor agonist exendin-4 improves reference memory performance and decreases immobility in the forced swim test, Eur. J. Pharmacol. 650 (2011) 249–255.
[235] M.J. During, L. Cao, D.S. Zuzga, J.S. Francis, H.L. Fitzsimons, X. Jiao, R.J. Bland, M. Klugmann, W.A. Banks, D.J. Drucker, C.N. Haile, Glucagon-like peptide-1 receptor is involved in learning and neuroprotection, Nat. Med. 9 (2003) 1173–1179.
[236] T. Abbas, E. Faivre, C. Hölscher, Impairment of synaptic plasticity and memory formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease, Behav. Brain Res. 205 (2009) 265–271.
[237] A.J. Kastin, V. Akerstrom, Entry of exendin-4 into brain is rapid but may be limited at high doses, Int. J. Obes. Relat. Metab. Disord. 27 (2003) 313–318.
[238] W.A. Banks, M.J. During, M.L. Niehoff, Brain uptake of the glucagon-like peptide-1 antagonist exendin(9–39) after intranasal administration, J. Pharmacol. Exp. Ther. 309 (2004) 469–475.
[239] T. Perry, D.K. Lahiri, D. Chen, J. Zhou, K.T. Shaw, J.M. Egan, N.H. Greig, A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells, J. Pharmacol. Exp. Ther. 300 (2002) 958–966.
[240] Y. Li, T. Perry, M.S. Kindy, B.K. Harvey, D. Tweedie, H.W. Holloway, K. Powers, H. Shen, J.M. Egan, K. Sambamurti, A. Brossi, D.K. Lahiri, M.P. Mattson, B.J. Hoffer, Y. Wang, N.H. Greig, GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 1285–1290.
[241] Y. Li, D. Tweedie, M.P. Mattson, H.W. Holloway, N.H. Greig, Enhancing the GLP-1 receptor signaling pathway leads to proliferation and neuroprotection in human neuroblastoma cells, J. Neurochem. 113 (2010) 1621–1631.
[242] T. Iwai, S. Ito, K. Tanimitsu, S. Udagawa, J. Oka, Glucagon-like peptide-1 inhibits LPS-induced IL-1β production in cultured rat astrocytes, Neurosci. Res. 55 (2006) 352–360.
[243] S. Kim, M. Moon, S. Park, Exendin-4 protects dopaminergic neurons by inhibition of microglial activation and matrix metalloproteinase-3 expression in an animal model of Parkinson's disease, J. Endocrinol. 202 (2009) 431–439.
[244] Y. Hattori, T. Jojima, A. Tomizawa, H. Satoh, S. Hattori, K. Kasai, T. Hayashi, A glucagon-like peptide-1 (GLP-1) analogue, liraglutide, upregulates nitric oxide production and exerts anti-inflammatory action in endothelial cells, Diabetologia 53 (2010) 2256–2266.
[245] T. Perry, N.H. Greig, Enhancing central nervous system endogenous GLP-1 receptor pathways for intervention in Alzheimer's disease, Curr. Alzheimer Res. 2 (2005) 377–385.
[246] B. Martin, E. Golden, O.D. Carlson, P. Pistell, J. Zhou, W. Kim, B.P. Frank, S. Thomas, W.A. Chadwick, N.H. Greig, G.P. Bates, K. Sathasivam, M. Bernier, S. Maudsley, M.P. Mattson, J.M. Egan, Exendin-4 improves glycemic control, ameliorates brain and pancreatic pathologies, and extends survival in a mouse model of Huntington's disease, Diabetes 58 (2009) 318–328.
[247] D.P. Bradley, R. Kulstad, D.A. Schoeller, Exenatide and weight loss, Nutrition 26 (2010) 243–249.
[248] J. Tong, D.A. Sandoval, Is the GLP-1 system a viable therapeutic target for weight reduction? Rev. Endocrinol. Metab. Disord. 12 (2011) 187–195.
[249] D.J. Good, Extending the reach of exendin-4: new pathways in the control of body weight and glucose homeostasis, Endocrinology 153 (2012) 2051–2053.
[250] D. Cummings, J. Overduin, Gastrointestinal regulation of food intake, J. Clin. Invest. 117 (2007) 13–23.
[251] P.S. Dalvi, A. Nazarians-Armavil, M.J. Purser, D.D. Belsham, Glucagon-like peptide-1 receptor agonist, exendin-4, regulates feeding associated neuropeptides in hypothalamic neurons in vivo and in vitro, Endocrinology 153 (2012) 2208–2222.
[252] N. Pannacciulli, J.C. Bunt, J. Koska, J. Bogardus, J. Krakoff, Higher fasting plasma concentrations of glucagon-like peptide 1 are associated with higher resting energy expenditure and fat oxidation rates in humans, Am. J. Clin. Nutr. 84 (2006) 556–560.
[253] A. Shalev, J.J. Holst, U. Keller, Effects of glucagon-like peptide 1 (7–36 amide) on whole-body protein metabolism in healthy man, Eur. J. Clin. Investig. 27 (1997) 10–16.
[254] H. Yamamoto, C.E. Lee, J.N. Marcus, T.D. Williams, J.M. Overton, M.E. Lopez, A.N. Hollenberg, L. Baggio, C.B. Saper, D.J. Drucker, J.K. Elmquist, Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons, J. Clin. Invest. 110 (2002) 43–52.
[255] R. Nogueiras, D. Perez-Tilve, C. Veyrat-Durebex, D.A. Morgan, L. Varela, W.G. Haynes, J.T. Patterson, E. Disse, P.T. Pfluger, M. López, S.C. Woods, R. DiMarchi,

C. Diéguez, K. Rahmouni, F. Rohner-Jeanrenaud, M.H. Tschöp, Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity, J. Neurosci. 29 (2009) 5916–5925.

[256] M.J. Perley, D.M. Kipnis, Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects, J. Clin. Invest. 46 (1967) 1954–1962.

[257] A.A. Young, B.R. Gedulin, S. Bhavsar, N. Bodkin, C. Jodka, B. Hansen, M. Denaro, Glucose-lowering and insulinsensitizing actions of exendin-4: studies in obese diabetic ((ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (Macaca mulatta)), Diabetes 48 (1999) 1026–1034.

[258] J. Eng, W.A. Kleinman, L. Singh, G. Singh, J.P. Raufman, Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom, J. Biol. Chem. 267 (1992) 7402–7405.

[259] J.P. Fortin, J.C. Schroeder, Y. Zhu, M. Beinborn, A.S. Kopin, Pharmacological characterization of human incretin receptor missense variants, J. Pharmacol. Exp. Ther. 332 (2010) 274–280.

[260] M.R. Hayes, T.M. Leichner, S. Zhao, G.S. Lee, A. Chowansky, D. Zimmer, B.C. De Jonghe, S.E. Kanoski, H.J. Grill, K.K. Bence, Intracellular signals mediating the food intake-suppressive effects of hindbrain glucagon-like peptide-1 receptor activation, Cell Metab. 13 (2011) 320–330.

[261] B.J. Kim, J. Zhou, B. Martin, O.D. Carlson, S. Maudsley, N.H. Greig, M.P. Mattson, E.E. Ladenheim, J. Wustner, A. Turner, H. Sadeghi, J.M. Egan, Transferrin fusion technology: a novel approach to prolonging biological half-life ofinsulinotropic peptides, J. Pharmacol. Exp. Ther. 334 (2010) 682–692.

[262] K.A. Al-Barazanji, J.R. Arch, R.E. Buckingham, M. Tadayyon, Central exendin-4 infusion reduces body weight without altering plasma leptin in (fa/fa) Zucker rats, Obes. Res. 8 (2000) 317–323.

[263] C.E. Pritchett, A. Hajnal, Glucagon-like peptide-1 regulation of carbohydrate intake is differentially affected byobesogenic diets, Obesity (Silver Spring) 20 (2012) 313–317.

[264] E.D. Baraboi, D.H. St-Pierre, J. Shooner, E. Timofeeva, D. Richard, Brain activation following peripheral administration of the GLP-1 receptor agonist exendin-4, Am. J. Physiol. Regul. Integr. Comp. Physiol. 301 (2011) R1011–R1024.

[265] J.G. Lim, J.J. Lee, S.H. Park, J.H. Park, S.J. Kim, H.C. Cho, W.K. Baek, D.K. Kim, D.K. Song, Glucagon-like peptide-1 protects NSC-34 motor neurons against glucosamine through Epac-mediated glucose uptake enhancement, Neurosci. Lett. 479 (2010) 13–17.

[266] L.R. Ranganath, J.M. Beety, L.M. Morgan, J.W. Wright, R. Howland, V. Marks, Attenuated GLP-1 secretion in obesity: cause or consequence? Gut 38 (1996) 916–919.

[267] T.C. Adam, M.S. Westerterp-Plantenga, Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects, Br. J. Nutr. 93 (2005) 845–851.

[268] R. Morínigo, V. Moizé, M. Musri, A.M. Lacy, S. Navarro, J.L. Marín, S. Delgado, R. Casamitjana, J. Vidal, Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects, J. Clin. Endocrinol. Metab. 91 (2006) 1735–1740.

[269] C. Holdstock, B. Zethelius, M. Sundbom, F.A. Karlsson, B. Edén Engström, Postprandial changes in gut regulatory peptides in gastric bypass patients, Int. J. Obes. (Lond.) 32 (2008) 1640–1646.

[270] J.P. Gutzwiller, B. Göke, J. Drewe, P. Hildebrand, S. Ketterer, D. Handschin, R. Winterhalder, D. Conen, C. Beglinger, Glucagon-like peptide-1: a potent regulator of food intake in humans, Gut 44 (1999) 81–86.

[271] E. Naslund, B. Barkeling, N. King, M. Gutniak, J.E. Blundell, J.J. Holst, S. Rössner, P.M. Hellström, Energy intake and appetite is suppressed by glucagon-like peptide-1 (GLP-1) in obese men, Int. J. Obes. 3 (1999) 304–311.

[272] M.C. Washington, S.J. Raboin, W. Thompson, C.J. Larsen, A.I. Sayegh, Exenatide reduces food intake and activates the enteric nervous system of the gastrointestinal tract and the dorsal vagal complex of the hindbrain in the rat by a GLP-1 receptor, Brain Res. 1344 (2010) 124–133.

[273] V. Darsalia, S. Mansouri, H. Ortsäter, A. Olverling, N. Nozadze, C. Kappe, K. Iverfeldt, L.M. Tracy, N. Grankvist, A. Sjöholm, C. Patrone, Glucagon-like peptide 1 receptor activation reduces ischaemic brain damage followingstroke in Type 2 diabetic rats, Clin. Sci. (Lond.) 122 (2012) 473–483.

[274] M.C. Bunck, M. Diamant, A. Cornér, B. Eliasson, J.L. Malloy, R.M. Shaginian, W. Deng, D.M. Kendall, M.R. Taskinen, U. Smith, H. Yki-Järvinen, R.J. Heine, One-year treatment with exenatide improves beta-cell function, compared with insulin glargine, in metformin-treated type 2 diabetic patients: a randomized, controlled trial, Diabetes Care 32 (2009) 762–768.

[275] O.G. Kolterman, D.D. Kim, L. Shen, J.A. Ruggles, L.L. Nielsen, M.S. Fineman, A.D. Baron, Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus, Am. J. Health Syst. Pharm. 62 (2005) 173–181.

[276] S. Teramoto, N. Miyamoto, K. Yatomi, Y. Tanaka, H. Oishi, H. Arai, N. Hattori, T. Urabe, Exendin-4, a glucagon-like peptide-1 receptor agonist, provides neuroprotection in mice transient focal cerebral ischemia, J. Cereb. Blood Flow Metab. 31 (2011) 1696–1705.

[277] C. Tourrel, D. Bailbe, M. Lacorne, M.J. Meile, M. Kergoat, B. Portha, Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the beta-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4, Diabetes 51 (2002) 1443–1452.

[278] D.C. Klonoff, J.B. Buse, L.L. Nielsen, X. Guan, C.L. Bowlus, J.H. Holcombe, M.E. Wintle, D.G. Maggs, Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years, Curr. Med. Res. Opin. 24 (2008) 275–286.

[279] R. Fan, X. Li, X. Gu, J.C. Chan, G. Xu, Exendin-4 protects pancreatic beta cells from human islet amyloid polypeptide-induced cell damage: potential involvement of AKT and mitochondria biogenesis, Diabetes Obes. Metab. 12 (2010) 815–824.

[280] A.H. Barnett, J. Burger, D. Johns, R. Brodows, D.M. Kendall, A. Roberts, M.E. Trautmann, Tolerability and efficacy of exenatide and titrated insulin glargine in adult patients with type 2 diabetes previously uncontrolled with metformin or a sulfonylurea: a multinational, randomized, open-label, two-period, crossover noninferiority trial, Clin. Ther. 29 (2007) 2333–2348.

[281] Y. Li, K.B. Duffy, M.A. Ottinger, B. Ray, J.A. Bailey, H.W. Holloway, D. Tweedie, T. Perry, M.P. Mattson, D. Kapogiannis, K. Sambamurti, D.K. Lahiri, N.H. Greig, GLP-1 receptor stimulation reduces amyloid-beta peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer's disease, J. Alzheimers Dis. 19 (2010) 1205–1219.

[282] G. Bertilsson, C. Patrone, O. Zachrisson, A. Andersson, K. Dannaeus, J. Heidrich, J. Kortesmaa, A. Mercer, E. Nielsen, H. Rönnholm, L. Wikström, Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease, J. Neurosci. Res. 86 (2008) 326–338.

[283] A. Harkavyi, A. Abuirmeileh, R. Lever, A. Kingsbury, C. Biggs, P.S. Whitton, Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease, J. Neuroinflammation 5 (2008) 19.

[284] T. Perry, H.W. Holloway, A. Weerasuriya, P.R. Mouton, K. Duffy, J.A. Mattison, N.H. Greig, Evidence of GLP-1-mediated neuroprotection in an animal model of pyridoxine induced peripheral sensory neuropathy, Exp. Neurol. 203 (2007) 293–301.

[285] P.L. McClean, V. Parthsarathy, E. Faivre, C. Hölscher, The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease, J. Neurosci. 31 (2011) 6587–6594.

[286] T. Perry, N.J. Haughey, M.P. Mattson, J.M. Egan, N.H. Greig, Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4, J. Pharmacol. Exp. Ther. 302 (2002) 881–888.

[287] T. Perry, D.K. Lahiri, K. Sambamurti, D. Chen, M.P. Mattson, J.M. Egan, N.H. Greig, Glucagon-like peptide-1 decreases endogenous amyloid-beta peptide (Abeta) levels and protects hippocampal neurons from death induced by Abeta and iron, J. Neurosci. Res. 72 (2003) 603–612.

[288] F. Calara, K. Taylor, J. Han, E. Zabala, E.M. Carr, M. Wintle, M. Fineman, A randomized, open-label, crossover study examining the effect of injection site on bioavailability of exenatide (synthetic Exendin-4), Clin. Ther. 27 (2005) 210–215.

[289] V.A. Gault, W.D. Porter, P.R. Flatt, C. Holscher, Actions of exendin-4 therapy on cognitive function and hippocampal synaptic plasticity in mice fed a high-fat diet, Int. J. Obes. 34 (2010) 1341–1344.

[290] M.A. Nauck, S. Duran, D. Kim, D. Johns, J. Northrup, A. Festa, R. Brodows, M. Trautmann, A comparison of twice-daily exenatide and biphasic insulin aspart in patients with type 2 diabetes who were suboptimally controlled with sulfonylurea and metformin: a non-inferiority study, Diabetologia 50 (2007) 259–267.

[291] D.J. Drucker, J.B. Buse, K. Taylor, D.M. Kendall, M. Trautmann, D. Zhuang, L. Porter, for the DURATION-1 Study Group, Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: a randomised, open-label, non-inferiority study, Lancet 372 (2008) 1240–1250.

[292] A.M. Delamater, Improving patient adherence, Clin. Diabetes 24 (2006) 71–77.

[293] K.S. Ingersoll, J. Cohen, The impact of medication regimen factors on adherence to chronic treatment: a review of the literature, J. Behav. Med. 31 (2008) 213–224.

[294] D. Russell-Jones, Molecular, pharmacological and clinical aspects of liraglutide, a once-daily human GLP-1 analogue, Mol. Cell. Endocrinol. 297 (2009) 137–140.

[295] S.E. Arnold, B.T. Hyman, J. Flory, A.R. Damasio, G.W. Van Hoesen, The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease, Cereb. Cortex 1 (1991) 103–116.

[296] W.N. Han, C. Hölscher, L. Yuan, W. Yang, X.H. Wang, M.N. Wu, J.S. Qi, Liraglutide protects against amyloid-β protein induced impairment of spatial learning andmemory in rats, Neurobiol. Aging 34 (2013) 576–588, (Electronic publication ahead of print).

[297] K. Hunter, C. Holscher, Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis, BMC Neurosci. 13 (2012) 33–38.

[298] R.D. Brinton, J.M. Wang, Therapeutic potential of neurogenesis for prevention and recovery from Alzheimer's disease: allopregnanolone as a proof of concept neurogenic agent, Curr. Alzheimer Res. 3 (2006) 185–190.

[299] M. Blurton-Jones, M. Kitazawa, H. Martinez-Coria, N.A. Castello, F.J. Muller, J.F. Loring, T.R. Yamasaki, W.W. Poon, K.N. Green, F.M. Laferla, Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 13594–13599.

[300] D.W. Porter, B.D. Kerr, P.R. Flatt, C. Holscher, V.A. Gault, Four weeks administration of Liraglutide improves memory and learning as well as glycaemic control in mice with high fat dietary-induced obesity and insulin resistance, Diabetes Obes. Metab. 12 (2010) 891–899.

[301] M. Christensen, F.K. Knop, T. Vilsboll, J.J. Holst, Lixisenatide for type 2 diabetes mellitus, Expert Opin. Investig. Drugs 20 (2011) 549–557.

[302] W.D. Porter, P.R. Flatt, C. Hölscher, V.A. Gault, Liraglutide improves hippocampal synaptic plasticity associated with increased expression of Mash1 in ob/ob mice, Int. J. Obes. (Lond.) (2012), http://dx.doi.org/10.1038/ijo.2012.91. (Jun 5, Electronic publication ahead of print).

[303] S.E. Kanoski, S.M. Fortin, M. Arnold, H.J. Grill, M.R. Hayes, Peripheral and central GLP-1 receptor populations mediate the anorectic effects of peripherally administered GLP-1 receptor agonists, liraglutide and exendin-4, Endocrinology 152 (2011) 3103–3112.

[304] L.L. Baggio, D.J. Drucker, Biology of incretins: GLP-1 and GIP, Gastroenterology 132 (2007) 2131–2157.

[305] A. Vella, P. Shah, A.S. Reed, A.S. Adkins, R. Basu, R.A. Rizza, Lack of effect of exendin-4 and glucagon-like peptide-1-(7,36)-amide on insulin action in non-diabetic humans, Diabetologia 45 (2002) 1410–1415.