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REVIEW ARTICLE

Intranasal Insulin for Alzheimer's Disease

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Abstract
Brain insulin signaling contributes to memory function and might be a viable target in the prevention and treatment of memory impairments including Alzheimer's disease. This short narrative review explores the potential of central nervous system (CNS) insulin administration via the intranasal pathway to improve memory performance in health and disease, with a focus on the most recent results. Proof-of-concept studies and (pilot) clinical trials in individuals with mild cognitive impairment or Alzheimer's disease indicate that acute and prolonged intranasal insulin administration enhances memory performance, and suggest that brain insulin resistance is a pathophysiological factor in Alzheimer's disease with or without concomitant metabolic dysfunction. Intranasally administered insulin is assumed to trigger improvements in synaptic plasticity and regional glucose uptake as well as alleviations of Alzheimer's disease neuropathology; additional contributions of changes in hypothalamus-pituitary-adrenocortical axis activity and sleep-related mechanisms are discussed. While intranasal insulin delivery has been conclusively demonstrated to be effective and safe, the recent outcomes of large-scale clinical studies underline the need for further investigations, which might also yield new insights into sex differences in the response to intranasal insulin and contribute to the optimization of delivery devices to grasp the full potential of intranasal insulin for Alzheimer's disease.

1 Introduction: Insulin in the Brain

Almost 50 million people worldwide lived with dementia in 2015 according to estimates based on over 200 studies, with expected increases to 75 million by 2030 and 132 million by 2050 [1]. Recent assumptions that the incidence and prevalence of dementia may remain stable or even decline offer a glimmer of hope [2], but the high total number of afflicted people and the severity of dementia-associated impairments in the daily life of patients and their families underline the magnitude of the challenge, which also poses a considerable financial burden on global health systems (estimated to have amounted to US$818 billion in 2015 [3]). Alzheimer's disease (AD) is the major cause of dementia, but there are still no causal treatments for this debilitating disease (cholinesterase inhibitors and memantine are used for symptomatic relief at early stages). The progressive loss of cognitive and functional abilities in AD is associated with the accumulation of aberrant, misfolded, and aggregated oligomeric amyloid beta (Aβ) peptides and hyperphosphorylated tau, but the etiology of AD remains poorly understood [4].

Recent research indicates that insulin action in the brain might be a key factor in its pathogenesis as well as a target of interventions to prevent and treat this devastating ailment.

Key Points

Insulin acting in the brain is a relevant neuromodulator that contributes to cognitive function via mechanisms that are still to be fully explored.

CNS insulin delivery via the nose improves memory performance in healthy individuals but also patients with Alzheimer's disease who are assumed to be less sensitive to the brain insulin signal.

Mixed results of larger scale clinical trials call for further research on the preconditions and mechanisms of the memory effect of intranasal insulin as well as for the optimization of delivery approaches.

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Although compared with other fields of neuroscience, central nervous system (CNS) insulin signaling is a relatively young topic, the last 30 years have greatly advanced our understanding of the mechanisms and functions of insulin's role in the brain and for the brain. The presence of insulin receptors in rat brains was first demonstrated by Havrankova et al. in 1978 [5]; not much later, insulin receptors were also detected in the human brain [6]. Insulin concentrations in cerebrospinal fluid (CSF) and plasma are correlated, but insulin concentrations are much lower in CSF [7]. It is assumed that the bulk of brain insulin has its source in peripheral insulin crossing the blood–brain barrier (BBB) by a saturable receptor-mediated transport mechanism [8]. Some indicators of local insulin production in the cerebral cortex have been found in animals [9, 10] and there are reports of insulin transcription in human brain tissue [11], but the assumption that insulin is released in decisive amounts within the brain still lacks coherent evidence [12].

As the brain does not essentially rely on insulin to regulate its energy supply [13, 14], the function of CNS insulin receptors first remained elusive; today, it is known that the neuropeptide affects a broad range of functions including peripheral energy and glucose homeostasis [15, 16], growth [17] and, notably, neuronal plasticity [18]. Stephen Woods and his team were the first to perform seminal studies showing that insulin, which circulates within the bloodstream in proportion to body fat stores, acts in the brain to reduce food intake [19]. This finding was repeatedly replicated [20, 21] and insulin is now regarded as an important adiposity signal that provides feedback from the body periphery to CNS circuitries that control energy intake [22]. Unsurprisingly for a signal of such obvious relevance for metabolism, research activities first focused on this aspect of brain insulin signaling. In the meantime, however, it has become clear that insulin's CNS function pertains to cognitive processes, suggesting that brain insulin action also constitutes a neuroendocrine link between metabolism and cognition and might be a suitable target in the treatment both of metabolic and cognitive disorders [23].

In patients with obesity and/or type 2 diabetes mellitus, who experience variable degrees of peripheral insulin resistance (i.e., a decrease or lack of effective insulin signaling), the brain is likewise less sensitive to insulin, which supports the notion that relative brain insulin resistance or a lack of insulin in the CNS is a key factor in dysfunctional metabolic control [24]. As will be discussed in this review, it is likely that impaired brain insulin sensitivity moreover contributes to memory impairments including AD; the potential of insulin in the prevention and therapy of AD is illustrated by evidence that insulin delivery to the CNS improves cognitive function in healthy individuals and, moreover, patients with cognitive impairments or AD.

In this regard, the intranasal (IN) approach to increase the availability of insulin in the CNS is of particular interest because it has been put to successful use in most of the more recent investigations that this narrative review focuses on. The search strategy pivoted around PubMed results in English language with the terms "intranasal", "brain", "insulin", "cognition", "memory", and "AD" retrieved until September 2020 and the reference lists of the respective publications, with a focus on work published since 2017. Note that the relevance of brain insulin signaling (and respective beneficial effects of IN insulin) pertains to neurological and psychiatric conditions such as vascular cognitive impairment [25], Parkinson's disease [26, 27], traumatic brain injury [28], Huntington's disease [29, 30], depression [31], and addictive behavior [32], which are outside the scope of the present paper.

2 Intranasal Insulin Administration to the CNS

The BBB, an endothelial layer of cells and tight junctions, separates the vessels perfusing the CNS from its environment, thereby shielding the brain against toxins and infections while allowing gas and ion exchange. It regulates the entry and exit of molecules into and out of the brain and, moreover, serves as a communication interface that is endowed with receptors and transporters for hormonal signals including insulin [33]. The BBB is passively permeable to molecules of approximately < 400 Da in size and with fewer than eight to ten hydrogen bonds; in addition, it enables the active, often saturable transport of bigger molecules [34]. With a molecular weight of 5808 Da, insulin is too large to cross the BBB passively and therefore depends on active transport mechanisms to enter the brain [35]. Insulin concentrations in the CSF increase after intravenous infusion in men [7], but the efficiency of blood-to-CSF transport is limited by conditions such as increases in body weight [36]. In experiments in animals, insulin is routinely administered to the CNS by, for example, direct intracerebroventricular [19] or hypothalamic infusion [37]. Systemic insulin administration to investigate CNS effects of the hormone has long been the method of choice in experiments in humans [e.g., 38–41], but this approach comes with some important drawbacks. The decrease in blood glucose concentrations induced by systemic insulin infusion below certain thresholds inevitably impairs cognition [42] and, moreover, activates endocrine (stress) axes that can affect brain function [43]. Insulin-induced hypoglycemia can be prevented by simultaneous glucose infusion that, however, may itself exert a biasing impact on (cognitive) brain functions. Euglycemic–hyperinsulinemic clamps are moreover time and labor intensive and do not permit the differentiation between direct brain effects and effects mediated via peripheral pathways.

The IN route of insulin administration overcomes these methodological impediments. The first US patent on IN administration to bypass the BBB and target the brain was filed by William H. Frey II in 1989 [44], followed by a second patent on IN insulin to treat AD and Parkinson's disease [45] and proof-of-concept demonstrations in animals [e.g., 46–48]. Experiments in Sprague-Dawley rats relying on gamma counting and high-resolution phosphor imaging of tissue sections suggest that after IN administration insulin-like growth factor-1 quickly activates multiple sites within the brain and spinal cord [48]. It has likewise been shown that intranasally administered neuropeptides reach brain structures that are relevant for cognitive function [49]. Considering that the intra-neuronal transport of neuropeptides from the nasal cavity to the olfactory bulb takes several hours [50], it is assumed that intranasally administered peptides primarily travel along extra-neuronal routes, i.e., through intercellular clefts of the olfactory epithelium situated on the superior turbinate and opposite the nasal septum [51, 52]; additional transport along trigeminal nerve branches to brainstem regions has been demonstrated [48, 53]. Studies in humans indicate that intranasally administered insulin can bypass the BBB and reach the CNS within 1 h after administration [54]. Systemic absorption after IN insulin administration is negligible at moderate doses [54] and seems to trigger side effects such as increases in cortisol and growth hormone only when cumulative doses exceed around 200 IU [55]. Therefore, it is also unlikely that BBB transport after absorption into the bloodstream is a major contributor to brain uptake and functional impact of IN insulin. The IN pathway moreover extends insulin's half-life by minimizing hepatic first-pass elimination [56]. It may also be possible to target specific areas of the brain, especially those near the administration site [57]. Because of its easy methodology and favorable safety profile [58] (see Sect. 4.3), the IN method of insulin administration to the brain offers a non-invasive, easy-to-use approach that has now been widely applied in experimental settings of preclinical but also clinical research (for in-depth reviews on the IN administration of insulin and other peptides see, for example, [52, 59, 60]). Indeed, it seems that besides oxytocin [61], insulin is the hormone with the most promising evidence of functional effectivity after IN delivery.

3 Intranasal Insulin and Memory

3.1 Intranasal Insulin-Induced Memory Improvements in Humans Without Cognitive Impairments

Beneficial cognitive effects of CNS insulin administration via the IN route have been demonstrated in a series of studies in healthy humans [62–66]. Eight weeks of IN insulin administration (4 × 40 IU/day vs diluent) to young men and women [63] improved the delayed recall of a list of 30 words encoded 1 week earlier, a measure of hippocampus-dependent declarative memory. In contrast, immediate word recall 3 min after encoding and non-declarative memory functions remained unaffected [63]. The improvement in declarative memory could even be intensified by administering the rapid-acting insulin analog insulin aspart [64]; insulin aspart has a reduced tendency to self-associate but shares the receptor binding profile of regular insulin [67]. In acute paradigms, preliminary evidence for sex-dependent insulin effects on memory function was obtained because women, but not men, improved performance on declarative and working memory tasks after receiving 160 IU of insulin compared to placebo (diluent) [62]. In subsequent experiments, IN insulin in comparison to placebo (diluent) administration before nocturnal sleep tended to improve the acquisition of word-pairs on the subsequent evening in women, with opposite effects in men [65]. In an acute experiment that only included healthy male participants [66], IN insulin compared with placebo (diluent) enhanced the odor-cued recall of spatial memory, while an impairing effect of IN insulin (vs diluent) on olfactory sensitivity was observed in young healthy women but not men [68]. Although experimental indicators of a preponderance of metabolic effects of IN insulin in men rather than women [62, 69] buttress the assumption of a sex difference in the functional response to IN insulin, studies in larger samples of male and female participants with cognitive impairments have only yielded sporadic evidence [70]. Animal experiments suggest that insulin's CNS impact is modified by estrogen signaling [71], but related studies in humans do not support the assumption that estrogen may boost the sensitivity to the memory effect of insulin [72]. Systematic investigations into sex differences in brain insulin effects, underlying mechanisms, or possible implications for the prevention and treatment of AD are currently lacking. This is somewhat surprising considering that the age-specific prevalence of AD is higher in women [1]—in the USA, two thirds of individuals with AD are women [73]—and that the risk of AD in carriers of the ε4 variant of the apolipoprotein E gene (apoE ε4), a risk factor for sporadic AD [74] whose frequency does not differ between men and women, is four times higher in women than men aged between 65 and 75 years [75] (for further AD-related sex differences, see [76]). Moreover, women have a greater risk of developing systemic insulin resistance [77].

In accordance with the results in normal-weight individuals [63], obese men who were administered IN insulin compared with placebo (diluent) for 8 weeks according to the same paradigm likewise displayed improvements in declarative memory [78]. Electrophysiological evidence for the impact of IN inulin on brain function was obtained

in experiments relying on magnetoencephalography [79] or measuring scalp-recorded event-related [80] and direct current brain potentials [81]. In the latter study, a largely comparable negative shift in direct current potentials was observed within minutes after IN and intravenous bolus administration of insulin that was assumed to reflect changes in extracellular ionic concentrations due to glial activity [82]. These findings suggest a rapid effect of insulin on brain activity in humans and, moreover, that IN insulin delivery is able to mimic the brain impact of intravenously administered and, presumably, endogenous insulin.

Brain insulin may not only modulate cognitive but also emotional functions. The 8-week paradigm of IN insulin administration induced an improvement in self-rated rated mood in normal-weight [63] as well as obese participants [78]. In mice, IN insulin enhances object memory and induces anxiolytic behavioral effects [83], whereas lentivirus-mediated downregulation of hypothalamic insulin receptor expression in rats elicits depressive and anxiety-like behaviors [84]. Impaired glucose tolerance due to diet-induced obesity likewise abrogates the memory-improving and anxiolytic impact of insulin [83]. Taken together, these findings suggest that impaired CNS insulin signaling might contribute to the association between metabolic disorders such as obesity and diabetes and cognitive impairments as well as dysphoria [85].

3.2 Mechanisms of the Enhancing Effect of Insulin on Cognition

In-vitro studies and experiments in animals, but also humans, have enabled insights into a number of possible mechanisms behind the improving cognitive impact of (intranasal) insulin. Insulin activates its receptor by binding to extracellular α-subunits and triggering the dimerization of intracellular β-subunits, thereby inducing receptor autophosphorylation. The two most relevant signaling pathways activated by insulin are the insulin-insulin receptor substrate (IRS)-Akt pathway (recruiting IRS1 or IRS2) and the mitogen-activated protein kinase pathway. The insulin-IRS-Akt pathway mediates the glucoregulatory action of insulin in muscle, adipose, and liver tissue and further downstream processes in all cell types, while the mitogen-activated protein kinase pathway regulates transcription factors such as CREB and c-Fos (see [86] for details). Brain insulin receptors are expressed in high densities in the olfactory bulb, hypothalamus, and cerebellum and in regions that enable memory formation such as the hippocampus and connected limbic brain structures [87, 88]. Neuronal insulin receptors are expressed both pre- and post-synaptically and neuronal insulin signaling relies on the insulin-IRS-Akt as well as the mitogen-activated protein kinase pathway [89]. Insulin has been demonstrated to contribute to a broad range of neuronal signaling mechanisms, including but not limited to catecholamine release and uptake, ion channel trafficking, and the regulation of receptors for neurotransmitters, i.e., γ-aminobutyric acid, *N*-methyl-D-aspartate (NMDA), and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) [90]. It also contributes to activity-dependent processes of synaptic plasticity, i.e., long-term potentiation and long-term depression [91]. More details on insulin signaling pathways in the brain can be found elsewhere [86, 92].

The establishment of memory traces in the hippocampus depends both on long-term depression and long-term potentiation [93]. Supporting the assumption that insulin improves memory by modulating these plastic processes, insulin was found to induce glutamatergic AMPA receptor internalization leading to long-term depression [94], and moreover to phosphorylate AMPA receptors leading to overexpression of PKMζ [95]. The downregulation of hippocampal insulin receptor function impairs long-term potentiation and spatial memory [96]. Insulin also potentiates NMDA receptor activity via delivery of NMDA receptors to the cell surface [97] and NMDA receptor phosphorylation [98], processes that may induce long-lasting meta-plastic changes. In addition to effects on synaptic plasticity [99], there is some evidence that insulin benefits regional brain glucose uptake by activating the neuronal glucose transporter type 4 and enhances glycogen uptake in regions such as the basal forebrain, hippocampus, amygdala, and cortex [100, 101] (for reviews see [102, 103]), in particular under conditions of high cognitive demand [104, 105]. In experiments in healthy humans relying on ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) measurements during intravenous insulin infusion while endogenous insulin production was suppressed by somatostatin, whole-brain glucose utilization was found to be stimulated by insulin [106], whereas experiments using ^{1}H-magnetic resonance spectroscopy yielded no effect of insulin infusion on brain glucose [14]. Neuronal glucose uptake is mostly regulated via glucose transporter type 3, which is generally assumed not to depend on insulin [107, 108]; however, recent in vitro experiments indicate that 4 days of insulin receptor activation up-regulate glucose transporter type 3 membrane expression in hippocampal neurons [109]. Insulin may also support brain energy supply via effects on astrocytes [110] and other glia cells including oligodentrocytes (for a review, see [86]). Moreover, IN insulin has been found to improve regional vasoreactivity alongside visuospatial memory function [111]. On a systems level, IN insulin administration was observed to increase the concentrations of high-energy phosphate compounds, i.e., adenosine triphosphate and phosphocreatine, in the motor cortex as assessed by ^{31}P-magnetic resonance spectroscopy, an effect that was positively related to the subsequent insulin-induced suppression in food intake [112]. Intranasal insulin can moreover trigger enhancements

in functional connectivity between prefrontal regions and the hippocampal formation that benefit memory formation [113].

Recent evidence points to sleep- and stress-related mechanisms as further potential mediators of improving cognitive effects of IN insulin. The impact on hypothalamic-pituitary-adrenal (HPA) axis activity of 160 IU of IN insulin administered before sleep was assessed in a study that included young and elderly healthy men and women [114]. In comparison with the young participants, the elderly subjects showed signs of increased cortisol concentrations during early sleep, when HPA axis secretion typically reaches its circadian nadir. Intranasal insulin compared to placebo (diluent) dampened cortisol levels in the first night-half in the elderly, but not in the young participants in a sex-independent manner. Reductions in HPA axis activity upon IN insulin vs placebo (diluent) were also observed in awake young men exposed to a psychosocial stress test [115], as well as under resting conditions after 8 weeks of daily administration [63, 78]. Attenuating effects of brain insulin on HPA axis activity have been assumed to be mediated by enhanced corticosteroid feedback processing in the hippocampus [116]. In healthy elderly humans, cortisol was found to acutely reduce FDG-PET-assessed glucose utilization in the hippocampus [117], and increases in HPA axis activity are associated with an increased risk for metabolic and cognitive impairments including AD [118–120]. It is of particular interest that insulin may co-regulate HPA axis activity in association with circadian and sleep-related mechanisms because the consolidation of memory content strongly benefits from sleep: neuronal ensembles that encode information during wakefulness are reactivated during subsequent sleep, thereby strengthening respective memory representations [121]. Accordingly, impaired sleep may predispose to or accelerate cognitive impairments including AD [122].

While IN insulin delivery before sleep does not affect polysomnographically assessed sleep architecture or subjective sleep quality [114], electroencephalogram delta power during the second 90 min of non-rapid-eye-movement (NREM) sleep was found to be enhanced by insulin compared with a placebo (diluent) in young healthy men [65]. Nocturnal insulin secretion is entrained to NREM sleep phases [123]. In rats, peripheral and intracerebroventricular administration of insulin increases the time spent in NREM sleep [124], whereas the hormone seems to have the opposite effect on REM sleep [124]. The enhancement of electroencephalogram delta power by IN insulin coincided with a pronounced, but statistically unrelated insulin-induced increase in growth hormone levels that was independent of the participant's sex [65]. Participants also encoded declarative and procedural memory contents (word-pairs and, respectively, finger tapping sequences) before IN insulin administration in the evening. Insulin compared to placebo did not directly alter the retrieval of memory contents acquired before sleep, but generally impaired the acquisition of interfering memory contents on the next day (although, as described above, the female participants displayed a trend to improved learning of new word-pairs in the insulin vs placebo condition). These results suggest that sleep-associated memory consolidation may not be a primary mediator of insulin's acute memory-improving effect in healthy subjects. Still, that IN insulin reduces the interfering influence of encoding new information on the subsequent day may be taken as an indicator that processes of active forgetting during sleep [125] are inhibited by insulin. Insulin-induced improvements in sleep electroencephalogram delta power may support the clearance of metabolic waste that is linked to slow-wave activity [126]; notably, slow-wave activity during NREM sleep has also been found to be negatively correlated with tau pathology and Aβ deposition in the brain of cognitively healthy aging humans [127].

A role for sleep-related mechanisms in cognitive improvements due to IN insulin would also be in line with observations in healthy male subjects that longer term daily IN administration of 160 IU of insulin vs placebo before nocturnal sleep, but not in the morning, induces slight improvements in declarative memory, i.e., delayed recall of words learned 1 week earlier [128], which also suggests that timing might be a critical determinant of IN insulin effects. This effect appeared to be more pronounced after 5 weeks compared with the end of treatment after 8 weeks, but all in all remained rather modest; interestingly, post-hoc median-split analyses suggested that participants with relatively high compared with those with relatively low systemic insulin sensitivity (reflected by homeostatic model assessment insulin resistance) benefitted to a greater extent [128]. While the mechanisms described in this paragraph are assumed to mediate the functional impact of boosting the physiological brain insulin signal in healthy adults, additional mechanisms likely come into play in individuals who exhibit impairments in memory performance, not least because such impairments are assumed to stem from reduced CNS insulin sensitivity.

4 Intranasal Insulin and Impaired Memory

4.1 Intranasal Insulin Effects in Humans with Mild Cognitive Impairment and AD

Interest in the role of brain insulin signaling in the development of AD and in methods to improve insulin action in the CNS to prevent disease progression has intensified in recent years [e.g., 129–131]. This interest has been stoked by pioneering studies conducted by Suzanne Craft and colleagues indicating that the beneficial effects of IN insulin

on declarative memory outlined above are not restricted to healthy participants but can also be found in people with mild cognitive impairment (MCI) or (early) AD (see [132] for a systematic review covering relevant research up to October 2017).

In a study in 23 men and women with AD and 14 aged-matched healthy controls who were all non-diabetic, intravenous insulin in comparison with placebo improved story recall, a measure of declarative memory function, and selective attention assessed with the Stroop interference test [133]. Subsequent trials made use of the IN paradigm. In a comparison of 13 adult men and women with early AD and 13 men and women with MCI, matched with 35 controls, the acute effect of IN insulin was investigated in three conditions (placebo [saline], 20 IU and 40 IU of insulin administered 15 min before cognitive assessments) [134]. The cognitive test battery assessed verbal declarative memory (story recall and word-list recall), visual working memory (self-ordered pointing task), selective attention (Stroop test), and visual search. Intranasal insulin compared with placebo improved both measures of recall only in memory-impaired apoE ε4-negative participants, whereas healthy controls did not benefit and memory-impaired apoE ε4 carriers even showed signs of insulin-induced deterioration of word-list recall. Follow-up studies found comparable patterns: apoE ε4-negative participants with memory impairments benefited from acute IN insulin vs placebo (saline) delivery in terms of memory improvement whereas apoE ε4 carriers demonstrated a relative decline [135]. Adults with MCI including amnestic symptoms (e.g., due to AD) who were treated with IN insulin for 3 weeks (2×20 IU/day, $n = 13$) showed significantly increased story recall compared with participants treated with a placebo (saline; $n = 12$) [136]. The observation of apoE ε4-dependent differences in the impact of IN insulin raises the possibility that brain insulin signaling may only be impaired, and therefore a particularly worthwhile target of interventions, in patients without the apoE ε4 allele [137], which has received further support in subsequent trials [41, 70] (for conflicting data see e.g., 138).

In a pilot clinical trial lasting 4 months [139], women and men diagnosed with MCI or mild-to-moderate AD received 40 IU of regular insulin, placebo (saline), or 40 IU of insulin detemir (each $n = 12$), a long-acting insulin analog with relatively high lipophilicity that has been assumed to exert stronger effects on brain functions than regular insulin [138, 140]. Cognitive tests included delayed story recall, the Alzheimer Disease Assessment Scale-cognitive subscale 12 (ADAS-Cog-12 [141]), and the Dementia Severity Rating Scale [142]. Intranasal delivery of regular insulin compared with placebo improved memory scores after 2 and 4 months of treatment and was associated with preserved magnetic resonance imaging (MRI)-assessed brain volumes in the left superior parietal cortex, right middle cingulum, left cuneus, and right parahippocampal gyrus. Surprisingly, insulin detemir administration remained without effects. In a related 4-month trial [143], male and female adults with amnestic MCI or mild-to-moderate AD received placebo (saline; $n = 30$) or 20 IU ($n = 36$) or 40 IU ($n = 38$) of regular insulin/day. In comparisons with the placebo group, story recall after a delay of 20 min was enhanced in the 20-IU but not in the 40-IU group, while caregiver-rated functional ability was preserved in both insulin-treated groups; moreover, the progression of hypometabolism assessed via FDG-PET was dampened in both insulin groups. Findings like these suggest that there may be an optimal regimen of IN insulin administration between doses that are too low and, notably, too high, i.e., a inverted U-shaped function of beneficial insulin effects. This assumption has received support in acute experiments by Suzanne Craft's group [135] and might imply that above a certain threshold (which is yet to be identified) insulin may impair cognitive function, potentially by inducing inflammatory effects (see Sect. 5) [144].

The results of the first multi-site phase II/III clinical trial of IN insulin for MCI and AD, conducted at 27 sites of the Alzheimer's Therapeutic Research Institute and including 289 participants (155 of them men) between 55 and 85 years of age with a diagnosis of amnestic MCI or AD, have been recently published [145]. The ViaNase device (Kurve Technology), which had been effectively used in previous studies on IN insulin [138, 139, 143], proved unreliable in the first 49 participants because of problems with a newly added electronic timer. Therefore, the remaining 240 participants (designated the primary intention-to-treat population) received a daily dose of 40 IU of insulin or placebo (diluent) with the I109 Precision Olfactory Delivery device (Impel NeuroPharma) for 12 months followed by a 6-month open-label extension phase. Mean score change on the ADAS-Cog-12 [141], evaluated at 3-month intervals, was the primary outcome measure. In contrast to the promising effects discussed above, no differences between insulin and placebo were observed in the primary measure or in other clinical (e.g., Alzheimer Disease Cooperative Study Activities of Daily Living Scale for MCI, ADLMCI [146]) or CSF parameters (e.g., Aβ42 and Aβ40, total tau protein, tau p-181, CSF insulin concentrations). Very small reductions in hippocampal and entorhinal cortex volume were identified by MRI in the insulin- compared with the placebo-treated participants. Interestingly, in secondary analyses of the participants who used the ViaNase device, signs of improved ADAS-Cog-12 scores were observed in the insulin ($n = 23$) compared with the placebo group ($n = 22$) during the blinded as well as during the open-label extension phase along with increased Aβ42–Aβ40 and Aβ42 to total tau ratios as well as an insulin-induced decrease in enthorinal cortex volume. Considering that the participants were allowed to receive background therapy such as cholinesterase inhibitors or memantine,

these improvements have been judged to be clinically relevant [25].

4.2 Brain Insulin Resistance in AD and Related Memory Impairments

Given that the CNS administration (via the IN pathway) of insulin, a major factor in the control of peripheral glucose homeostasis, ameliorates cognitive function in amnestic patients, it is not surprising that impairments in systemic and brain insulin sensitivity have been found to be interrelated and that they may jointly contribute to the pathogenesis and progression of AD. "Brain insulin resistance," defined as the failure of brain cells to respond to insulin [24, 86], on a functional level implies that the CNS insulin signal does not effectively support cognitive processes (or the control of metabolism), and could involve downregulation or failure of insulin receptors as well as impairments of downstream signaling. Brain insulin resistance may be a cooccurrence or, potentially, a consequence of peripheral insulin resistance, which is for example in line with Fernanda de Felice's cumulative hypothesis that the additive impact of unhealthy lifestyles (e.g., low physical activity, inadequate nutrition) eventually results in defects of brain metabolism and brain insulin signaling that trigger cognitive decline [92]. Notably, impairments in peripheral insulin signaling in individuals with AD were suggested more than 25 years ago [147]. Frazier and colleagues have recently come up with an inspiring account of research into brain insulin resistance, putting forward the idea that whereas brain insulin signaling may be impaired in AD, type 2 diabetes, and aging, insulin sensitivity per se may be preserved in these conditions [103]. Indicators of brain insulin resistance have also been found in the relative absence of systemic insulin resistance (see below). As pointed out recently [25], however, it is unclear whether insulin resistance can develop in the brain independently from systemic insulin resistance. Additionally, brain insulin resistance so far has only been determined in relation to supposedly normal insulin effects on the brain, whereas discrete functional, neurophysiological, or neuroimaging-derived criteria have not been established [25]. A number of cognitive domains have been consistently observed to be affected in individuals with type 2 diabetes (e.g., memory, psychomotor speed, executive function, processing speed, verbal fluency, attention [137]) and respective organ deficits include white matter lesions [148] as well as ischemic impairments, cerebral atrophy, and cortical hypometabolism [86]. In animal experiments, chronic hyperinsulinemia as found in obesity and diabetes was demonstrated to decrease the number of insulin receptors at the BBB [35], thereby attenuating brain insulin uptake. Aggregation of advanced glycation end-products due to hyperglycemia likewise compromises BBB functionality [149]. Such impairments might contribute to the increased incidence of AD in patients with metabolic impairments like diabetes that is indicated by epidemiological as well as experimental findings [e.g., 150, 151] (for reviews see [152, 153]), and that may have unfavorable therapeutic consequences when it comes to diabetes self-management [154]. A recently completed clinical trial (NCT02415556) has investigated the impact of long-term administration (24 weeks and 24 weeks follow-up) of IN insulin (40 IU/day vs saline) on measures of cognition (e.g., spatial working memory, paired associate learning), daily functionality, and gait speed in adults with type 2 diabetes and controls of aged 50–85 years [155]; its results are expected to potentially identify clinical phenotypes that predict the response to IN insulin.

Using high spatial resolution, arterial spin labeling MRI at rest and during mild hypercapnia, Frosch and colleagues [156] compared lean controls and obese or overweight adults with and without insulin resistance and found a reduction in cerebrovascular reactivity to mild hypercapnia in obesity compared with normal weight. In the obese subjects with insulin resistance, cerebrovascular reactivity and insulin sensitivity as reflected by QUICKI values [157] were significantly related, suggesting that impairments in cerebrovascular reactivity might precede full-blown diabetes and eventually result in a vicious circle of central and peripheral insulin resistance. Notably, individuals with systemic insulin resistance also display a decrease in hippocampal volume [158] and hippocampal atrophy, a marker of neurodegeneration [159]. Hyperphosphorylated tau in CSF and brain parenchyma [160, 161] and increased deposition of Aβ [162, 163] have been found to be associated with signs of insulin resistance in some studies. Although this and related evidence [164] points to an association of systemic insulin resistance or type 2 diabetes and molecular symptoms of neurodegenerative diseases, many studies have failed to establish such a relationship [e.g., [165] (for in-depth discussions of in-vivo and post-mortem studies as well as genetic risk factors, see [25, 86]). Recent investigations that assessed brain Aβ accumulation via ^{11}C-Pittsburgh compound B (PiB)-PET scans in 41 individuals with type 2 diabetes of the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) likewise revealed only weak indicators of a relationship between blood markers of insulin resistance and Aβ deposition [166].

There is some experimental support for the assumption that brain insulin resistance may contribute to the development of AD independent of systemic failures in insulin signaling (as in type 2 diabetes) [e.g., 92, 167–169]. Post-mortem analyses of the brains of patients with AD have indicated decreases in messenger RNA and protein expression of insulin and insulin receptors as well as insulin-like growth factor-1 and insulin-like growth factor-2 along with signs of reduced downstream insulin signaling mechanisms that were

related to disease markers of AD [167]. Such changes may trigger negative consequences for neuronal repair, dendritic sprouting, and differentiation [170] and impair neuronal plasticity via detrimental effects on glutamatergic and cholinergic pathways [137, 171]. In subsequent and very sophisticated analyses of post-mortem hippocampal tissues from elderly individuals with or without AD, without a history of diabetes, indicators of dysregulation of insulin signaling pathways were detected [168]: in a novel ex vivo stimulation paradigm, insulin signaling cascades were strongly impaired in the hippocampal tissue of patients compared with controls matched for age and sex, and these impairments were negatively related to scores of cognition and memory. In further post-mortem analyses of insulin signaling in the middle frontal gyrus cortex in 150 individuals (mean age at death, 87 years, 48% women), there were no differences between individuals with or without diabetes in IRS1 phosphorylation (pS^{307}IRS1/total IRS1) and Akt phosphorylation (pT^{308}Akt1/total Akt1); the latter was highly significantly associated with composite scores of AD pathology [172]. (In contrast to the previous findings from the same group [168], IRS1 serine phosphorylation was not found to be associated with cognitive AD pathology in this sample.) The concentration of insulin in the CSF of patients with AD appears to be an unresolved issue as some reports have indicated increased [173] or, on the contrary, reduced levels [174, 175], whereas other findings point to normal concentrations [176, 177]; the respective contribution of potential impairments in insulin production within the CNS is an intriguing, albeit debated issue [9, 11, 86, 103]. Deteriorations in the clearance and degradation of Aβ due to insulin resistance are discussed as a mechanism that increases the risk of AD [178] and may be improved by insulin administration [179–181]. In 3×Tg-AD mice, a rodent model of AD, IN insulin compared with placebo administration for 2 months improved measures of short-term memory (spatial learning in the Morris water maze test and novel object recognition), ameliorated depressive-like behavior (assessed by the tail suspension and the forced swim test), and decreased markers of disease pathology, i.e., tau phosphorylation in the hippocampus and frontal cortex as well as hippocampal concentrations of Aβ oligomers and 3-nitrotyrosine [182]. These findings extend previous observations in animal experiments (e.g., [83, 183]). Brain insulin resistance has also been assumed to be influenced by genetic factors in addition to and beyond apoE ε4. For example, subjects with the FTO gene polymorphism rs8050136 as well as carriers of the Gly972Arg polymorphism of IRS1 exhibit a decreased cerebrocortical response to intravenous insulin [184, 185].

4.3 Effectiveness and Safety of Intranasal Insulin for AD

Only one study so far has presented straightforward evidence for CSF uptake of insulin after IN delivery in humans [54]. Although studies in animals conclusively support the assumption that IN administered substances (including insulin) are readily transported to the brain compartment [59], further experimental corroboration of the bioavailability of IN insulin, not least in patients with AD and related disorders as well as elderly individuals, would be welcome evidence for the effectiveness of IN insulin delivery. Nevertheless, respective experiments on other peptides such as oxytocin [186] corroborate the feasibility of IN peptide administration. Considering the lack of effects on primary outcome measures in the recent multi-site phase II/III clinical trial of IN insulin for MCI and AD [145], the currently available devices for IN drug delivery may benefit from further optimization [187]. The device used in that trial, which relies on a liquid hydrofluoroalkane propellant to eject a metered dose of insulin through a nose tip and achieved very high adherence rates, had not been previously tested in patients with AD but proved effective in animal experiments [59]. In this context, it should be noted that CSF increases after IN delivery of insulin [54] and a plethora of functional effects [62–65, 69, 72, 81, 114, 128, 188] in humans were observed in experiments that used a simple spray atomizer to initiate nose-to-brain transport of insulin. (Pharmacokinetic considerations notwithstanding, the same can be said of IN oxytocin [189]). Thus, it seems worthwhile to ponder if delivery devices that include more advanced, but maybe less robust or reliable, hardware or electronic components are essential to achieve successful brain uptake of IN administered hormones. While specifically targeting the upper third of the nasal cavity to optimally reach the olfactory epithelium is certainly a worthwhile idea [59], functional MRI assessments of regional cerebral blood flow corroborate the effectiveness of basic nasal spray devices [190]. However, considering that advanced age [191] and cognitive impairments including AD [192] are associated with olfactory impairments that may be exacerbated by nasal membrane atrophy and nasal obstructions, efforts to improve the bioavailability of intranasally administered drugs are warranted. Relying on, for example, the use of nanoparticle carriers [193], cell-penetrating peptides [194], focused ultrasound [195], and other absorption enhancers [196], they have yielded promising results and might be expected to enhance the nasal uptake of insulin while maintaining the safety profile and low systemic exposure associated with IN administration.

Insulin treatment did not increase CSF insulin concentrations regardless of the administration device in the phase II/III trial, but the measurements were made at single timepoints during baseline and after 12 months of administration; the authors conclude that direct (CSF- or imaging-derived) proof of the ability of an IN device to target the CNS should best be collected before its use in clinical trials [145]. As a side note, it is worth mentioning a peculiar feature of IN insulin. All experiments in healthy participants and clinical cohorts described herein used insulin formulations (e.g., Novolin R, Humulin R, Levemir) that contain *m*-cresol (meta-cresol), an excipient with a distinct "coal tar" smell that is highly noticeable (and sometimes reported to be unpleasant) during IN use. In experiments with a crossover design [e.g., 63–65, 114, 128], it seems therefore mandatory to administer a diluent/carrier solution in the placebo condition to prevent premature unblinding. Although this precaution might appear of lesser relevance for parallel studies that expose participants to only one treatment [e.g., 70, 136, 138, 139, 143], it is conceivable that the intense smell of insulin solutions elicits stronger expectancy effects than a non-odorous placebo, with potential implications for cognitive outcomes (perhaps even in respective animal studies). In the recent phase II/III trial, this potential confounder was excluded by using a diluent for the placebo [145].

The principal effectiveness to enhance memory function of boosting brain insulin signaling by IN insulin delivery in healthy participants, but also individuals with MCI or AD has been demonstrated in the studies discussed above. While signs of a modulating effect of apoE-ε4 on the neurofunctional impact of IN insulin in patients with AD have been repeatedly found ([134, 135, 138, 139]; see above) and animal experiments hint at potentially underlying mechanisms [197], systematic investigations in humans are needed to clarify the relevance of apoE-ε4 in the response to IN insulin [198], also with regard to the role of brain glucose metabolism. Experiments relying on FDG-PET in middle-aged adults at risk of developing AD revealed an association between systemic insulin resistance and lower glucose metabolism in the left temporal medial lobe that predicted impaired immediate and delayed memory performance, but did not interact with apoE-ε4 status; however, carriers of one or two ε4 alleles displayed decreased global glucose metabolism [199]. Mice carrying the apoE ε4 variant in comparison with controls carrying the ε2 allele, which is assumed to be protective, show reduced BBB glucose transport [200], suggesting that the higher AD risk in carriers of apoE ε4 may in part derive from reduced glucose transport into the brain [201]. Against the background of these and related reports of impaired brain glucose metabolism in AD ([e.g. [202, 203]), it might be speculated that insulin-induced enhancements of cognitive function in memory-impaired patients that occur within minutes at least in part derive from increases in cerebral glucose metabolism. However, considering that the absence of apoE ε4 appears to be a prerequisite for the cognitive impact of IN insulin, additional glucose-independent mechanisms are likely; it has also been argued that enhanced glucose uptake may mediate the acute effects of IN insulin whereas prolonged treatment may be necessary to induce improvements in synaptic plasticity [204]. In recent analyses of plasma samples obtained before and after 4 months of IN insulin vs saline administration to participants with MCI [205], favorable cognitive outcomes (ADAS-Cog) in response to the 20-IU dose of IN insulin [143] were mirrored by changes in neuronal extracellular vesicle biomarkers of insulin resistance (pS312-IRS-1, pY-IRS-1), which are known to be increased in patients with type 2 diabetes or AD and discussed as an easily accessible marker of brain insulin resistance [25]. This outcome, which appeared to be restricted to apoE ε4 non-carriers, suggests the engagement of the neuronal insulin cascade.

A meta-analysis of the efficacy and acceptability of antidiabetic agents (IN insulin, pioglitazone, rosiglitazone, metformin, and liraglutide) for MCI and AD that comprised 19 studies published until January 2018 found that antidiabetic treatments overall improved cognitive performance [206]. Thus, approaches to overcome CNS insulin resistance might for example make use of the insulin-sensitizing effects of glucagon-like peptide-1 [207] or of metformin that is routinely prescribed for type 2 diabetes [208]. Metformin enhanced memory and decreased the concentrations of Aβ, hyperphosphorylated tau, and activated microglia in AD mouse models along with signs of improved insulin signaling in the brain [209, 210]. On the background of promising metformin effects on memory performance in individuals with MCI but without diabetes [211], a phase II trial (NCT04098666) in patients with MCI or AD is ongoing. While initial studies also boded well for the use of the peroxisome proliferator-activated receptor-γ agonist rosiglitazone [212], subsequent clinical trials did not indicate primary endpoint improvements in AD [213]. Moreover, a recent multi-site trial of piaglitazone in healthy participants aged 65 years or older with a high genotype-determined risk of developing cognitive impairments due to AD was terminated early for a lack of efficacy (NCT01931566 [214]). It should also be noted that lifestyle interventions to improve dietary habits [215] and increase physical activity [216] hold some promise to ameliorate cognitive impairments and AD, possibly via enhancements in brain insulin signaling.

The safety profile of IN insulin has been systematically reviewed [58] (see [132, 217] for further reports). In 38 studies on acute IN insulin administration that included 1092 participants, no adverse events or cases of hypoglycemia were reported. Eighteen studies used long-term administration, with durations between 21 days and 9.7 years and a combined number of 832 participants. The only

symptomatic case of hypoglycemia in these studies was reported after administration of a placebo spray [218]. It was concluded that irritation of the nasal mucosa is the most commonly reported side effect, and that the IN route for insulin administration is safe and well tolerated both during acute and chronic use. These findings were corroborated in related meta-analyses [206] and the most recent trial on IN insulin [145] that found no indicators of clinically relevant adverse events as a result of the daily administration of 40 IU of insulin with two different administration devices.

5 Concluding Remarks

Some caveats should be mentioned. Considering the hyperinsulinemia that accompanies peripheral insulin resistance, it might be argued that the (relative) reduction of CSF insulin observed in obese individuals [36] and, in some experiments, in patients with AD [174, 175], represents a protective mechanism limiting CNS hyperinsulinemia and potentially detrimental sequelae of cellular insulin resistance in CNS pathways. This speculative assumption is in line with the observations of dose-dependent effects of IN insulin administration on memory function discussed above: acute IN insulin administration to individuals with AD improved verbal memory recall at lower (20 IU) but not higher doses (up to 60 IU); in carriers of the apoE ε4 allele, higher doses were even found to compromise memory performance [135]. Acute moderate euglycemic hyperinsulinemia in healthy individuals has been found to increase markers of CNS inflammation and Aβ formation [144], both of which increase the risk to develop cognitive impairments. However, pro-inflammatory in vitro effects on glial cells were found to vanish at higher insulin concentrations [219] and IN insulin decreased neuroinflammation and hippocampal lesion volume in a rat model of traumatic brain injury [28] (see [220, 221] for a discussion of insulin signaling and inflammatory processes in neurodegenerative disorders). The assumption that CNS hyperinsulinemia might promote brain insulin resistance is supported by in vitro experiments indicating that prolonged (4–24 h) exposure of hypothalamic cells to high insulin concentrations inactivate and degrade insulin receptors and IRS-1 [222]. Therefore, and against the background of the outcomes of most recent larger trials [145], it will be critical to investigate if the beneficial effects of acute and prolonged IN insulin administration can be corroborated and eventually put to use in the clinical setting, or if exogenous insulin delivery implies the risk of "induced brain insulin resistance." Moreover, there are many open questions regarding the mechanisms underlying and the implications of impaired brain insulin signaling in cognitive and metabolic disorders. They concern the relationship between AD and diabetes and brain concentrations of insulin, the factors that mediate cognitive impairments in metabolic disorders and, not least, the question whether neurodegeneration in AD can negatively affect the CNS control of systemic energy metabolism and contribute to systemic insulin resistance [86].

With regard to the use of IN insulin to prevent or counteract neurodegenerative disorders, future research may focus on a number of unresolved major issues:

- Considering that (long-term) IN insulin delivery alone might be associated with gradual downregulation of CNS insulin sensitivity, may its combination with insulin sensitizers such as metformin be superior in boosting cognitive function? Should IN insulin be administered *after* improvements in (CNS) insulin sensitivity have been achieved in patients with cognitive impairments and metabolic comorbidities via conventional means such as lifestyle intervention, so that resulting gains in brain functions can be preserved?
- Which delivery approaches and devices are optimally suited to enable nose-to-brain transport of insulin and other drugs, particularly in the clinical setting? Which absorption enhancers are best equipped to maximize brain permeation of IN insulin, and which doses, dosing schedules, insulin formulations, or insulin analogs are needed for the optimization of the memory effect?
- To which extent do mechanisms related to olfaction and sensory perception contribute to memory improvements after IN insulin delivery? Do sleep-related and circadian neurophysiological and neuroendocrine processes and stress-related psychoneuroendocrine factors modulate the impact of IN insulin in a (clinically) relevant manner?
- Does the cognitive (as well as metabolic) response to IN insulin critically depend on age and sex, and if so, how can future treatment approaches relying on IN insulin be tailored to the individual needs of patients?

In sum, while the bulk of experimental work outlined in this review underlines the effectiveness of IN insulin to improve memory function, there is still some work to be done to avoid pitfalls and fulfill the potential of IN insulin for AD.

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