Outcomes and clinical implications of intranasal insulin administration to the central nervous system

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Abstract word count: 181
Word count: 6880

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Insulin signaling in the brain plays a critical role in metabolic control and cognitive function. Targeting insulinergic pathways in the central nervous system via peripheral insulin administration is feasible, but associated with systemic effects that necessitate tight supervision or countermeasures. The intranasal route of insulin administration, which largely bypasses the circulation and thereby greatly reduces these obstacles, has now been repeatedly tested in proof-of-concept studies in humans as well as animals. It is routinely used in experimental settings to investigate the impact on eating behavior, peripheral metabolism, memory function and brain activation of acute or long-term enhancements in central nervous system insulin signaling. Epidemiological and experimental evidence linking deteriorations in metabolic control such as diabetes with neurodegenerative diseases imply pathophysiological relevance of dysfunctional brain insulin signaling or brain insulin resistance, and suggest that targeting insulin in the brain holds some promise as a therapy or adjunct therapy. This short narrative review gives an overview over recent findings on brain insulin signaling as derived from human studies deploying intranasal insulin, and evaluates the potential of therapeutic intervention that target brain insulin resistance.

Keywords: Intranasal insulin; central nervous system insulin signaling; metabolic control; cognitive function; memory; diabetes; obesity; Alzheimer's disease.

1. Insulin and the brain

Insulin is best known for its role in the peripheral control of blood glucose concentrations, but recent years have witnessed the discovery of many insulin effects that go beyond glycemic regulation. Animal studies have shown that insulin has important central functions (Vogt and Brüning, 2013; Kleinridders et al., 2014), not only in regard to metabolic control, but also to the regulation of eating behavior and even cognitive function. That the brain exerts some control over glucose homeostasis and metabolism has been known since the work of Claude Bernard in the 19th century (Bernard, 1855), although the respective mechanisms were unknown until the turn of this century (e.g., Brüning et al., 2000; Obici et al., 2002a; 2002b). Earlier on, in the late 1970s (Havrankova et al., 1978), insulin receptors were detected in the brain of rats. Since further studies showed that the brain itself does not rely on insulin to regulate its energy supply (Hom et al., 1984; Seaquist et al., 2001), the function of central nervous insulin receptors (Unger et al., 1991) first remained elusive, and there are still many questions to answer before a complete map of insulin actions in the brain can be drawn. One of the most important issues in this context (which is also at the focus of much current research) concerns the relevance of brain insulin signaling in patients with obesity and/or type 2 diabetes, who suffer from variable degrees of peripheral insulin resistance (i.e., a decrease or lack of effective insulin signaling): are their *brains* likewise less sensitive to insulin and do they display insufficient insulin uptake, and to which extent can therapeutic interventions improve or reverse such defects? A number of studies reviewed here suggest that relative brain insulin resistance or a lack of insulin in the CNS is indeed a key factor in dysfunctional metabolic control, and point towards therapeutic avenues to enhance peripheral metabolism and food intake behavior by targeting brain insulin signaling. Insulin administration to the central nervous system (CNS) moreover improves cognitive function in healthy adults, but

also patients with cognitive impairments such as Alzheimer's disease (AD). Findings like these gain particular relevance in light of evidence linking dysfunction in brain insulin signaling to neurodegenerative diseases such as AD, which has led to the suggestion that this devastating neurological disease may also be referred to as "type 3 diabetes" (Steen et al., 2005; Kandimalla et al., 2017).

We will summarize previous and most recent findings on the effects of CNS insulin administration in humans, with a focus on the intranasal pathway to increase the availability of insulin in the CNS (see Figure 1 for an overview). We aim at covering, in a narrative fashion, recent trends in research on the contribution of brain insulin to metabolism, energy turnover and ingestive behavior, and will also discuss advances regarding the use of insulin for studying and treating neurodegenerative disorders. We will pay particular attention to evidence that insulin modulates brain activity and behavior differentially in women and men (Hallschmid et al., 2004; Benedict et al., 2008; Feld et al., 2016), not least because of the obvious relevance of sex-specific effects when it comes to potential clinical applications.

2. The intranasal pathway to deliver insulin to the CNS

2.1 The blood-brain barrier

The blood-brain barrier (BBB) is an endothelial layer composed of multiple cells and tight junctions that separates the vessels perfusing the brain from the environment of the CNS. It shields the CNS against changes in hormonal signals, toxins, and infections, while allowing gas and ion exchange. By regulating the entry and exit of molecules from the blood into and out of the brain, it creates a stable environment for the brain to function. Additionally, far from isolating, the BBB serves as a communication platform because it is endowed with receptors and transporters for, e.g.,

metabolic signals like leptin, ghrelin, and insulin (Banks, 2001; 2012). It also provides mechanisms for the active transport of bigger molecules across the barrier, an often saturable process that connects the periphery and the brain mostly in a unidirectional manner. Moreover, the BBB is passively permeable to molecules of approximately <400 Da in size and with fewer than eight to ten hydrogen bonds (Pardridge, 2009). The endothelium regulates the transport of other molecules, and some states may alter this balance (e.g., obesity, starvation, hyperglycemia, AD; Banks, 2004). A common strategy for drug delivery to the brain is to modify the specific molecule by making it more lipophilic and thus more prone to be carried across the BBB. Another possibility is to take advantage of naturally present transporters.

Even though the limitations of blood-to-brain transport are known, many drugs targeting the CNS still fail in clinical trials (Gribkoff and Kaczmarek, 2017), which is true both for small molecule drugs and macromolecules (Ghosh et al., 2018). Manufacturers and researchers have focused on the molecules, trying to come up with modifications that improve their action while somewhat neglecting research into delivery mechanisms. Nonetheless, some manufacturers are working on interesting solutions, e.g., "piggybacking" on peptides. One of these approaches uses a modified antibody fragment to the transferrin receptor and improves transcytosis (Niewoehner et al., 2014). An efficient non-invasive method that up to now has stirred more interest in experimental researchers than pharmaceutical companies is the intranasal route.

2.2 Intranasal insulin administration

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 (Schwartz et al., 1990; Banks et al., 1997). Wallum and colleagues showed that insulin concentrations in cerebrospinal fluid (CSF) increase after intravenous infusion in men (Wallum et al., 1987). Other observations have

suggested a partial independence of extracelullar hypothalamic insulin from plasma levels (Gerozissis et al., 1997). It is also important to note that insulin concentrations are higher in plasma than in CSF, and that the ratio between CSF and plasma insulin is malleable; e.g., it decreases with increasing body weight (Kern et al., 2006). There is evidence that within narrow limits insulin is likewise secreted by neuronal subpopulations within the brain (Molnar et al., 2014), which may open additional avenues to increase insulin concentrations in the CNS (Csajbók and Tamás, 2016).

The nasal cavity is now known to be a direct gateway to the CNS (Gizurarson, 2012). The first U.S. patents on the intranasal administration mode were filed by William H. Frey II in 1989, and respective proof-of-concept studies in animals followed (for in-depth reviews, see Jogani et al., 2008; Crowe et al., 2018). Peptides and proteins can enter the brain directly or indirectly through contact with the cribriform plate (Meredith et al., 2015). (Indirect transport via absorption into the bloodstream is of course associated with the BBB-related hurdles described above.) Studies in Sprague-Dawley rats on brain delivery of insulin-like growth factor-I, which relied on gamma counting and high-resolution phosphor imaging of tissue sections, suggest that after intranasal administration this peptide quickly activates multiple sites within the brain and spinal cord (Thorne et al., 2004). These results support the assumption that after intranasal uptake, these and other hormones bypass the BBB along extracellular/paracellular olfactory and trigeminal pathways from the nasal mucosa to the subarachnoid space, followed by rapid transport via perivascular channels to targets throughout the brain (Crowe et al., 2018). The same group performed a similar experiment on interferon-beta in non-human primates and found that its administration through the nose quickly targeted a widespread range of central nervous sites (Thorne et al., 2008). Specifically, the olfactory bulb and trigeminal nerve exhibited significantly higher concentrations than peripheral organs and levels that were at least one order of magnitude

higher than those of any other sampled nervous tissue (Thorne et al., 2008). These and related findings indicate that macromolecules, when administered via the intranasal route, bypass the BBB and target the brain. Accordingly, Born and colleagues demonstrated in humans that the intranasal administration of insulin induces increases in CSF concentrations of the peptide within around 45 min (Born et al., 2002). It seems that the intranasal route is the current method of choice for insulin delivery to the brain in human experimental settings because of its easy methodology and favorable safety profile (Schmid et al., 2018; see below).

Importantly, intranasally administering peptides such as insulin reduces the overall systemic exposure, which in turn limits side effects. This pathway also minimizes exposure of the active compounds to hepatic first-pass elimination, the gut and blood (Djupesland et al., 2014; Gizurarson, 2012), which improves half-life. There is also evidence that it is possible to target specific areas of the brain (Falcone et al., 2014), especially areas near the administration site (Van de Bittner et al., 2018). For in-depth reviews on intranasal administration of peptides in general, please see Pires et al., 2009; Lochhead and Thorne, 2012; Kozlovskaya et al., 2014.

2.3 Safety and effectiveness

Recently, Schmid and colleagues have systematically reviewed the safety profile of intranasal insulin (Schmid et al., 2018; see Reger et al., 2006, and Gizurarson, 2012 for further reports). They reviewed 38 studies on acute intranasal insulin administration that included a total number of 1092 human participants and found that no adverse events or cases of hypoglycemia were reported. Eighteen studies dealt with chronic administration, with durations between 21 days and 9.7 years and a combined total number of 832 participants. Only one of these studies reported a symptomatic case of hypoglycemia, albeit after treatment with placebo spray (Kupila et al., 2003). Overall, the

authors conclude that irritation of the nasal mucosa is the most commonly reported side effect, and that the route is safe both when used acutely and chronically.

The effectiveness of the intranasal route is somewhat negatively affected by the distance between the entry point and the target in the brain, and relatively low bio-availability. Different research groups have focused on improving the latter and obtained promising results using PEGylation (Kim et al., 2012; Kamiya et al., 2018), cell-penetrating peptides (Khafagy et al., 2010; Kamei and Takeda-Morishita, 2015), and focused ultrasound (Chen et al., 2014; Ye et al., 2018). In general, these techniques improve uptake via the nose while maintaining the safety profile and low systemic exposure associated with "plain" intranasal insulin administration, i.e., its use without additives or changes.

A point of criticism is the low number of studies showing clear changes in CSF insulin availability after intranasal delivery – in fact, to the best of our knowledge, the only available report in humans is still the one by Born and coworkers (2002). There are, however, reports on other peptides such as oxytocin (Striepens et al., 2013) and vasopressin (Yang et al., 2012) that corroborate the principal effectiveness of intranasal peptide administration. The intranasal insulin doses usually used in experimental work range from 40 IU to 160 IU. At the lower-end of that range, elevations in CSF insulin concentrations of around 25 pmol can be expected, which is under the threshold necessary to activate insulin signaling in postmortem brains (see Talbot et al., 2012, for an in-depth discussion of this topic). Still, there is a growing body of evidence from behavioral and neuroimaging studies that clearly demonstrates that intranasally administered insulin modulates the activity of central pathways and peripheral organ systems.

3. CNS insulin in metabolic control

3.1 Food intake and body weight

The central nervous regulation of ingestive behavior is an intricate process (Morton et al., 2014), and the detailed description of its neuronal and neuroendocrine underpinnings is beyond the scope of this review. In direct and, respectively, indirect analogy to hormones such as the adipokine leptin (Münzberg and Morrison, 2015; Paz-Filho et al., 2015) and the gastrointestinally released ghrelin (Pradhan et al. 2013; Garin et al., 2013), insulin responds to feeding because its secretion peaks after food intake and might therefore act as a satiety signal that informs the brain about acute energy intake and contribute to the reduction in appetite (Stingl et al., 2010).

Studies in various animal models – dogs (Chen et al., 1975), baboons (Woods et al., 1979), rats (Brief and Davis, 1984) – provided early evidence that insulin, when administered directly to the brain, has the ability to reduce food intake and lower body weight. More recent research has continued to reproduce these data, with similar findings in chicks (Honda et al., 2007) and mice (Brown et al., 2006). The administration method in these studies was the intracerebroventricular route which, however, might involve a certain risk of unspecificity as suggested by comparable experiments that failed to detect anorexigenic insulin effects in rats (Jessen et al., 2010).

In humans, intranasal insulin administration for eight weeks (at a dose of 4 × 40 IU/d) reduced body weight and body fat content in men, but not in women (Hallschmid et al., 2004). The loss of body fat in the men was associated with a reduction of 27% in the levels of circulating leptin, while women experienced an increase of around 1 kg in body weight, mostly due to increased body-water. While actual food intake was not assessed in that study, subsequent acute experiments found a similarly sex-dependent pattern in the acute effect on ingestive behavior (Benedict et al., 2008). Fasted men ate less after intranasal insulin (160 IU) compared to placebo

administration, whereas women ate similar amounts of food in either condition. Interestingly, hippocampus-dependent memory and working memory performance was improved by intranasal insulin in women, but not men (see paragraph 4.2). The assumption of sex-specific effects of central insulin delivery on food intake is in accordance with animal studies (Clegg et al., 2003). In a later study by our group (Hallschmid et al., 2012), we administered intranasal insulin or placebo to healthy women after a standardized meal or, in control experiments, before standardized lunch intake. Insulin administered in the postprandial, but not in the fasted state reduced the intake and rated palatability of chocolate cookies offered some time after lunch (Hallschmid et al., 2012). These results imply that insulin delivery to the CNS curbs food intake also in women, if administered in a suitable time window, and that the hormone might act on reward-processing pathways that regulate "hedonic" eating motivation in the relative absence of hunger due to calorie depletion. The latter assumption is in line with more recent reports that intranasal insulin attenuates food-related activity in mesolimbic regions (Tiedemann et al., 2017).

While the study in women by Hallschmid et al. (2012) and the experiments by Tiedemann et al. (2017), which included men and women alike, do not support the assumption of decreased sensitivity to the anorexigenic impact of insulin in the female compared to the male organism, experiments in rodents suggest that systemic estrogen may decrease the food intake-related efficacy of central insulin (Clegg et al., 2006). However, postmenopausal women with comparatively reduced circulating estrogen do not differ in their susceptibility to the intranasal insulin effect from young women (on ethinyl estradiol-dominant contraceptives; Krug et al., 2010). Likewise, pretreating healthy young men with transdermal estrogen for three consecutive days – thereby strongly increasing their serum estradiol concentrations – does not attenuate the suppressive acute effect of intranasal insulin on calorie intake (Krug et al., 2018). Taken together,

these studies indicate that in humans, estrogen and insulin do not interact in the control of food intake, and leave open the question of sex-specific anorexigenic insulin effects, as does a recent experiment that investigated the impact of pre-sleep intranasal insulin administration (160 IU) on breakfast intake in male and female adults of young or higher age (Santiago and Hallschmid, 2017). Both the men and the women, irrespective of age, ate less from the standardized 4500 kcal breakfast buffet when they had received intranasal insulin compared to placebo before going to bed. This finding implies that an acute enhancement of the insulin signal in the CNS in the evening exerts a surprisingly extended hypophagic effect across sleep as a period of relative metabolic inactivity (see paragraph 4.4 for a discussion of sleep-related insulin effects). However, most recent data from our group (Ritze et al., unpublished data) indicate that when repeated daily for eight weeks, the pre-sleep administration paradigm does not reduce body weight in healthy men, suggesting that pre-meal delivery schedules as previously applied (Hallschmid et al., 2004) might be necessary to tap into the catabolic potential of intranasal insulin.

3.2 Brain networks and brain metabolism

In a study comprising a sample of ten lean and ten obese healthy volunteers (whose sex was not disclosed), Stingl and coworkers (2010) used magnetoencephalography to investigate the effect of 160 IU intranasal insulin or placebo on resting state network activity in the brain. They found an insulin-induced change of path length in the theta band (4-8 Hz) that differed between lean and obese participants and showed a positive linear correlation with BMI. According to the authors, this pattern can be interpreted as a decrease in global communication efficiency in obese subjects, possibly reflecting reduced insulin-triggered signaling between different brain regions involved in satiation and homeostatic control (Stingl et al., 2010). In related experiments using functional magnetic resonance imaging (fMRI), Kullmann and colleagues (2013) assessed resting-state brain

activity in lean, healthy women before and after application of intranasal insulin (160 IU) or placebo. Thirty and 90 min after insulin compared to placebo administration, the fractional amplitude of low-frequency fluctuations in the left orbitofrontal cortex and hypothalamus was decreased. These areas are critical for the regulation of food intake, especially with regard to the processing of its rewarding value (Dagher, 2010). Recently, that group also explored the effect of intranasal insulin on resting-state brain functional connectivity in healthy young adults (Kullmann et al., 2017). Twenty-five lean and 22 overweight or obese men and women underwent fMRI scans before and after receiving either intranasal insulin (160 IU) or placebo. Intranasal insulin acutely enhanced functional connectivity between prefrontal regions of the default-mode network and the hippocampus as well as the hypothalamus. Importantly, the authors observed the increase in hypothalamic functional connectivity only in participants with high as compared to those with low peripheral insulin sensitivity. The conclusion that reductions in peripheral insulin sensitivity go along with blunted insulin sensitivity of brain regions like the hypothalamus that are essential for metabolic control is in line with our previous observation that long-term administration of intranasal insulin does not reduce body weight in obese men (Hallschmid et al., 2008; see also paragraph 4.2).

In experiments relying on ^{31}P magnetic resonance spectroscopy in healthy fasted men, intranasal insulin administration at a dose of 40 IU increased brain energy levels, i.e., the concentrations of adenosine triphosphate and phosphocreatine, and subsequently reduced breakfast intake by around 12% (Jauch-Chara et al., 2012), suggesting that a surge in brain energy levels is a mediator of the hypophagic effect of intranasal insulin. In accordance with previous studies (Benedict et al., 2008; Santiago and Hallschmid, 2017), hunger ratings did not differ between conditions, supporting the conclusion that insulin delivery – and associated brain effects

– influence processes of satiation that contribute to the termination of a meal rather than the urge to eat. These findings on insulin effects on brain metabolism tie in with the concept that obesity is associated with chronic impairments in hypothalamic sensing of the brain's energy supply (Peters, 2011; Peters et al., 2007) and suggest that insulin delivery to the CNS has some potential to re-calibrate respective supply networks. (For an extensive account on CNS insulin effects as observed in neuroimaging studies, see Kullmann et al., 2016.)

3.3 Glucose homeostasis and whole-body energy metabolism

Against the background of animal studies indicating that peripheral glucose homeostasis partly depends on intact CNS (in particular hypothalamic) insulin signaling (Obici et al., 2002a; 2002b; Pocai et al., 2005), Ott and coworkers (2015) investigated the effect of intranasal insulin administration to the CNS on peripheral glucose homeostasis. High cumulative doses (up to 420 IU) of the insulin analog aspart were administered to healthy fasted men, a paradigm that enabled the differentiation between exogenous and endogenous insulin kinetics. While the repetitive intranasal insulin delivery reduced plasma glucose concentrations, small amounts of exogenous insulin also permeated into the circulation; in intravenous control experiments that mimicked this spillover, essentially identical changes in glucose homeostasis were observed, leaving open the question whether insulin delivery to the CNS affects peripheral glucose homeostasis in humans. Heni and colleagues (2017) used a hyperinsulinemic-euglycemic clamp with d-[6,6-$2H_2$] glucose infusion to measure endogenous glucose production and glucose disappearance, and on a separate day recorded fMRI to assess insulin-induced changes in regional cerebral blood flow. They found that after 160 IU of (regular human) intranasal insulin in comparison to placebo, lean volunteers needed a higher glucose infusion rate to maintain blood glucose levels, which appeared to be due to greater suppression of endogenous glucose production as well as stronger glucose uptake into

peripheral tissues. Spillover effects of intranasal insulin were controlled for, and the related fMRI findings suggested an involvement of hypothalamus and striatum in these effects which, notably, were absent in obese subjects. These outcomes are very much in line with the results of pancreatic clamps with a primed, constant infusion of glucose tracers that were performed in normal-weight (Dash et al., 2015) as well as overweight or obese subjects (Xiao et al., 2018). In these experiments, intranasal insulin (40 IU of insulin lispro) in comparison to placebo suppressed endogenous glucose production only in the normal-weight subjects. Although other experiments failed to detect insulin lispro in CSF after intranasal administration (Lowe et al., 2017), these results suggest that the reduction in peripheral insulin sensitivity seen in obese and overweight adults is associated with an impairment in the ability of CNS insulin to regulate peripheral glucose homeostasis, and lead to the question if brain insulin resistance might contribute to the pathogenesis of insulin resistance in obesity and diabetes (see paragraph 4.1).

Providing yet another indicator of overlapping or complementary functions of peripheral and CNS insulin signaling, Iwen and colleagues (2014) found evidence for a suppression of systemic lipolysis after intranasal administration of 160 IU to healthy men. The intervention reduced circulating free fatty acid concentrations and lipolysis (assessed via the appearance of deuterated glycerol), while fat biopsies indicated that lipolytic protein expression in subcutaneous adipose tissue was not affected. These preliminary results support the concept of a functional brain insulin-adipocyte axis (Koch et al., 2008). The influence of centrally administered insulin on energy expenditure was investigated in healthy normal-weight men by means of indirect calorimetry to measure postprandial thermogenesis (Benedict et al., 2011). Intranasal insulin (160 IU) in comparison to placebo increased diet-induced thermogenesis, an effect that might add to its catabolic impact via decreases in food intake (Benedict et al., 2008). In animals,

intracerebroventricular insulin administration moreover increases locomotor activity (Hennige et al., 2009), but it remains to be seen if similar effects can be induced in humans by means of intranasal insulin. The intranasal insulin-induced decrease in postprandial concentrations of circulating insulin and C-peptide observed in the thermogenesis study (Benedict et al., 2011) buttresses the assumption of respective improvements in peripheral insulin sensitivity (Dash et al., 2015; for review see Heni et al., 2015).

4. Dysfunctions in CNS insulin signaling as a link between metabolic and cognitive disorders

Obesity is associated with loss of gray matter and reduced integrity of white fiber tracts, especially within the limbic system (Kullmann et al., 2015), and diabetes negatively affects the brain via, e.g., vascular damage leading to lacunar infarcts and microbleeds (Brundel et al., 2014). Hippocampal atrophy, a marker of neurodegeneration, has been demonstrated in individuals with insulin resistance (Convit et al., 2003; Ursache et al., 2012). It is reasonable to assume that such changes contribute to the link between metabolic impairments and cognitive dysfunction that is evident from epidemiological as well as experimental findings (e.g., Cukierman et al., 2005; van Gemert et al., 2018; for reviews see Seaquist, 2015; Assuncao et al., 2018), and that may imply unfavorable therapeutic consequences when it comes to diabetes self-management (Punthakee et al., 2012). In recent years, evidence has accumulated that insulin resistance, a key symptom of metabolic disorders, is another major pathophysicological factor in cognitive impairments including AD; importantly, insulin resistance does not only occur in the body periphery, but also in the brain (e.g., Tschritter et al., 2006; for review see Kullmann et al., 2016).

4.1 Alzheimer's disease and insulin resistance

With a global prevalence as high as 35.6 million and numbers expected to increase even further in the near future (Prince et al., 2013), dementia is a major health issue. AD in particular is the source of tremendous suffering for afflicted individuals and their families, and poses an estimated burden of over $172 billion on worldwide healthcare systems (Alzheimer's Association, 2010). Progressive loss of cognitive and functional abilities as major symptoms of AD are associated with the accumulation of aberrant, misfolded and aggregated oligomeric amyloid-beta peptides and hyperphosphorylated tau (for review see Scheltens et al., 2016). Since the turn of the 21th century, researchers have shown growing interest in the role of CNS insulin signaling in AD (Benedict and Grillo, 2018; de la Monte, 2012; de Felice, 2013; Stanley et al., 2016), and the National Institutes of Health decided to allocate substantial funding to a trial on intranasal insulin as one of two therapeutic approaches for AD (Wadman, 2012; ClinicalTrials.gov identifier: NCT01767909).

The assumption that the pathogenesis of AD involves disrupted brain insulin signaling has received ample experimental support (e.g., de Felice et al., 2009; Rivera et al., 2005; Talbot et al., 2012; Pardeshi et al., 2017). Frosch and colleagues (2017) investigated this hypothesis in a study in 60 adult volunteers without cognitive impairments. They compared differences in cerebrovascular reactivity (CVR) to mild hypercapnia in lean controls and obese or overweight adults with and without insulin resistance. The results from high spatial resolution arterial spin labeling MRI at rest and during mild hypercapnia indicated that obesity compared to normal weight is associated with lower CVR. In obese subjects with insulin resistance, CVR and insulin sensitivity as reflected by QUICKI values (Katz et al., 2000) were significantly related ($r = 0.575$), implying that impairments in CVR might precede full-blown diabetes. Thus, improving insulin sensitivity in the brain could be a path to preserving its function and preventing age-associated cognitive decline. Accordingly, Fernanda de Felice's cumulative hypothesis poses that the additive

impact on peripheral organs of unhealthy life-styles (e.g., low physical activity, inadequate nutrition) eventually results in defective brain metabolism (de Felice, 2013). Deteriorations in clearance and degradation of amyloid-beta due to insulin resistance are discussed as another mechanism that increases the risk of AD (Craft, 2009). AD patients have also been reported to display decreased concentrations of insulin-receptor substrate-1 and insulin-like growth factor (Logan et al., 2018; Steen et al., 2005), with negative consequences for neurononal repair, dendritic sprouting and differentiation (Hölscher and Li, 2010). There is some debate on the concentration of insulin in the CSF of AD patients: early reports indicated increased (Fujisawa et al., 1991) or, on the contrary, reduced levels (Craft et al., 1998), while more recent findings point to normal concentrations (Geijselaers et al., 2017; Molina et al., 2002). In this context it is worth noting that chronic hyperinsulinemia as found in obesity and diabetes can decrease the number of insulin receptors at the BBB, thereby attenuating insulin transport into the brain and impairing central insulin action (Schwartz et al., 1990). Likewise, the amassment of advanced glycation end-products due to hyperglycemia negatively affects BBB functionality (Sasaki et al., 1998). Such impairments might contribute to the increased incidence of AD in patients with diabetes (Sims-Robinson et al., 2010).

Brain glucose metabolism has been reported to be decreased in the brains of middle-aged adults at risk for developing AD in experiments relying on F18-fluorodeoxyglucose positron emission tomography that also revealed an association between the decrease in glucose metabolic rate and impaired immediate and delayed memory performance (Willette et al., 2015). The ε4 variant of the Apo lipoprotein E (APOE) gene, a known risk factor for AD, also appears to influence brain metabolism. Mice carrying this APOE variant in comparison with controls carrying the ε2 allele, which is assumed to be protective, displayed reduced BBB glucose transport at twelve

but not four months of age (Alata et al., 2015). This pattern suggests that the higher AD risk in carriers of APOE ε4 may in part derive from chronically lower glucose transport into the brain (Verghese et al., 2011). Taken together, these findings raise the question whether insulin-related impairments in brain function can be improved by increasing the availability of insulin in the CNS.

4.2 Insulin-induced memory improvements in cognitively healthy subjects

The impact of intranasal insulin on memory function was first assessed in cognitively healthy young subjects (Benedict et al., 2004; 2007; 2008). Eight weeks of intranasal administration (4×40 IU/d) to young men and women (Benedict et al., 2004) improved delayed recall of a word list encoded one week earlier, a measure of hippocampus-dependent declarative memory, as well as self-rated mood. This improvement can even be boosted by applying the rapid-acting insulin analog insulin aspart (Benedict et al., 2007), which has a reduced tendency to self-associate (Brange and Volund, 1999) but shares the receptor binding profile of regular insulin (Kurtzhals et al, 2000). In acute experiments, sex-dependent insulin effects on memory were revealed, i.e., women, but not men, improved performance on declarative and working memory tasks after receiving 160 IU insulin compared to placebo (Benedict et al., 2008). Beneficial effects of insulin on memory function may be mediated via insulin receptors located in brain regions with relevance for memory formation, such as the hippocampus and connected limbic brain structures (Unger et al, 1991). Insulin can induce AMPA receptor internalization, which leads to long-term depression (Man et al, 2000), and also phosphorylates AMPA receptors and leads to the overexpression of PKMζ (Adzovic and Domenici, 2014). Accordingly, downregulating hippocampal insulin receptor function impairs long-term potentiation and spatial memory (Grillo et al, 2015). The establishment of memory traces in the hippocampus depends both on long-term depression and long-term potentiation (Goh and Manahan-Vaughan, 2015), so that insulin may exert some of its memory-

ehancing effects by modulating these plastic processes. The strong effect of estrogen on synaptic plasticity (Baudry et al, 2012) may interact with respective insulin effects and yield sex differences in insulin's memory effect (Benedict et al, 2008; Krug et al, 2010; Feld et al., 2016). Insulin also potentiates NMDA receptor activity (Liu et al, 1995) via delivery of NMDA receptors to the cell surface (Skeberdis et al, 2001) and NMDA receptor phosphorylation (Christie et al, 1999), processes that may induce long-lasting meta-plastic changes (Hulme et al, 2013). In addition to such synaptic effects, enhanced functional connectivity between prefrontal regions and the hippocampal formation due to intranasal insulin might benefit memory formation on a systems level (Kullmann et al., 2017).

In further experiments (Hallschmid et al., 2008), obese men were intranasally administered insulin for eight weeks according to the paradigm applied in normal-weight subjects (Benedict et al., 2004). In contrast to the results obtained in lean men, the obese insulin-treated participants did not lose body weight or fat. Nonetheless, in line with the normal-weight subjects they did show insulin-induced improvements in declarative memory and signs of improved mood. This pattern raises the intriguing question whether defective brain insulin signaling as a potential pathophysiological factor in metabolic and cognitive disorders can be more easily overcome (e.g., by intranasal insulin delivery) in the cognitive than metabolic domain; while this hypothesis has not yet been explicitly tested, promising results in memory-impaired subjects bode well for potential clinical uses of insulin delivery to the CNS.

4.3 Intranasal insulin delivery to the CNS as a therapeutic option

In a study in 23 non-diabetic men and women with AD and 14 aged-matched healthy, non-diabetic controls, intravenous insulin in comparison with placebo improved declarative memory function (as assessed by story recall) and selective attention (Stroop interference test; Craft et al., 1999).

Considering the technical and pharmacokinetic advantages of intranasal compared to intravenous insulin, subsequent trials made use of this paradigm to investigate the insulin effect in adults with mild cognitive impairment (MCI) or AD. Reger and co-workers (2006), examined 13 male or female adults with early AD and 13 men and women with MCI, matched with 35 male or female controls, in three conditions (placebo, 20 IU, 40 IU) taking place on separate mornings. 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. Memory-impaired patients without the APOE ε4 allele improved memory recall after intranasal insulin delivery, while those with APOE ε4 and healthy controls did not. Follow-up studies found a comparable pattern, with APOE ε4-negative patients benefiting most from insulin treatment in terms of memory improvement (Reger et al., 2008a; Reger et al., 2008b). The tentative conclusion that insulin resistance increases the risk for AD only in patients without APOE ε4 allele (Kodl and Seaquist, 2008) and that insulin administration might only be helpful in these patients, has received further support in the meantime (Craft et al., 2003; Alata et al., 2015; Verghese et al., 2011; Claxton et al., 2013; for conflicting data see Claxton et al., 2015).

In a recent pilot clinical trial of four months (Craft et al., 2017), women and men diagnosed with MCI or mild to moderate AD received 40 IU regular insulin, placebo, or 40 IU of insulin detemir, a long-acting insulin analog with relatively high lipophilicity. Cognitive tests included delayed story recall, a version of the Alzheimer's Disease Assessment Scale for Cognition, and the Dementia Severity Rating Scale. Intranasal administration of regular insulin induced improvements in memory after two and four months of treatment and was associated with preserved MRI-assessed brain volume in AD-related regions of interest, as well as reduction in the tau-P181/Aβ42 ratio in CSF (the latter measure was only obtained in subsets of patients).

Surprisingly, no significant effects were found in the insulin detemir group, and assessments of daily functioning did not show improvements in either group. In a related 4-months trial in male or female adults with amnestic mild cognitive impairment or mild to moderate AD, subjects receiving 20 IU or 40 IU of regular insulin/d compared with placebo-assigned participants showed reduced progression of hypometabolism in widespread brain regions during the 4-month treatment period, as assessed via F18-fluorodeoxyglucose positron emission tomography (Craft et al., 2012). These effects on brain glucose metabolism were found alongside memory improvements in the 20-IU but not the 40-IU group and preserved caregiver-rated functional ability in both groups. Evidence for the assumption of an optimal dose of intranasal insulin between too low and, notably, too high doses, i.e., a ∩-shaped function of beneficial insulin effects, also emerged in acute experiments by the same group (Reger et al., 2008a). These findings suggest that above a certain threshold (which has not yet been identified in respective experiments in healthy humans), insulin might impair cognitive function, potentially by inducing inflammatory effects in the CNS (Fishel et al., 2005).

4.4 Targeting stress axis activity and sleep physiology

Stress axis activity and sleep appear to be sensitive to insulin delivery to the CNS; although respective evidence may have received less attention than insulin's direct effects on metabolic control and memory function, we would like to argue that they might still gain relevance for potential clinical applications. Increases in hypothalamo-pituitary-adrenal (HPA) axis activity are associated with an increased risk for metabolic and cognitive impairments (McEwen, 2000; Incollingo Rodriguez et al., 2015; Popp et al., 2015). Likewise, sleep contributes to energy homeostasis and food intake regulation (St-Onge, 2013; Reutrakul and van Cauter, 2018):

habitually short sleep goes along with increased body weight (Magee and Hale, 2012; Vgontzas et al., 2014) and a greater risk of impaired glucose homeostasis (Gangwisch et al., 2007; Cappuccio et al., 2010). Moreover, the consolidation of memory contents markedly benefits from the brain's offline processing during sleep (Feld and Born, 2017) because neuronal ensembles that encode information during wakefulness are reactivated during subsequent sleep, thereby strengthening respective memory representations (Diekelmann and Born, 2010). Accordingly, impaired sleep is discussed as a risk factor for cognitive disorders including AD (Cedernaes et al., 2017).

The effect on HPA axis activity of intranasal insulin administration (160 IU) before sleep has been recently investigated in both young and elderly healthy men and women (Thienel et al., 2017). As expected, the elderly compared with the young participants displayed signs of elevated cortisol concentrations during early sleep (when HPA axis secretion reaches its circadian nadir). Intranasal insulin compared to placebo decreased cortisol concentrations in the first night-half in the elderly, but not the young subjects, and this effect and was independent of their sex. Acutely dampening effects of intranasal insulin on HPA axis activity have also been found in awake, young male subjects exposed to a psychosocial stress test (Bohringer et al., 2008), as well as under resting conditions after eight weeks of daily administration (Benedict et al., 2004). While results on changes in HPA axis activity and responsivity in patients suffering from post-traumatic stress disorder are not unanimous (Bremner et al., 2003; Wichmann et al., 2018; Schubert et al., 2019), intranasal insulin has already been discussed as a potential intervention to ameliorate this stress-related disorder (Frey, 2013). Attenuating effects of CNS insulin on HPA axis activity may be mediated by enhanced corticosteroid feedback processing in the hippocampus (de Kloet et al., 2018), which is assumed to exert inhibiting control over the HPA system via projections to the hypothalamus (Jacobson & Sapolsky, 1991). Notably, the respective effect of long-term intranasal

insulin delivery even kicked in earlier and was more pronounced in obese compared to normal-weight men (Hallschmid et al., 2008).

In the study by Thienel and coworkers (2017), neither sleep architecture measured with polysomnography nor subjective sleep quality was influenced by insulin administration. However, more fine-grained analyses of the results in the young subjects revealed that EEG delta power during the second 90 min of non-rapid-eye-movement sleep was enhanced by insulin in the male participants (Feld et al., 2016). Nocturnal insulin secretion is entrained to NonREM sleep phases (Kern et al, 1996), and peripheral and intracerebroventricular administration of insulin to rats increases the time spent in NonREM sleep (Danguir and Nicolaidis, 1984; Sangiah et al, 1982), whereas REM sleep appears to be reduced (Sangiah et al., 1982). The enhancement of EEG delta power, which coincided with a pronounced, but statistically unrelated insulin-induced increase in growth hormone concentrations that was independent of the sex of the subject, may have been due to insulin counteracting the homeostatic reduction of sleep pressure that normally manifests in waning delta band activity during the night (Borbely and Achermann, 1999). Interestingly, the EEG effect was absent in female subjects, which may have been related to estrogen that is known to alter sleep architecture and homeostatic regulation in rats (Deurveilher et al, 2011). In the study by Feld and colleagues (2016), subjects also encoded declarative and procedural memory contents before intranasal insulin administration in the evening (i.e., they learned word-pairs and trained on a finger-sequence tapping task). Insulin compared to placebo impaired the acquisition of new contents in both the declarative and procedural memory systems on the next day (the impairment in interference word-pair learning appeared to be restricted to the male subjects), whereas retrieval of original memories was unchanged. These findings indicate that sleep-associated memory consolidation may not be a primary mediator of insulin's acute memory-improving effect in

healthy subjects, but that the neuropeptide acts on mechanisms that diminish the subsequent encoding of novel information. Thus, by inhibiting processes of active forgetting during sleep (Hardt et al., 2013), insulin might reduce the interfering influence of encoding new information and thereby benefit memory formation.

5. Conclusions

Insulin influences a number of relevant brain functions, in particular those related to metabolic control and memory. (The scope of this review does not permit a full account of the multi-faceted nature of CNS insulin effects as derived from human and animal experiments; see e.g., Porte et al., 2005; Vogt and Brüning, 2013; Nguyen et al., 2018). It seems certain now that obesity, diabetes and cognitive impairments like AD imply pathophysiological – and probably also etiological – changes in CNS insulin signaling. While the topic gains more and more traction in basic and pre-clinical research, making therapeutic use of these insights beyond pilot trials still appears a long way off. A number of groups around the globe work on fulfilling the promise held by intranasal insulin administration to the CNS, as is also reflected by the review papers specifically devoted to intranasal insulin in 2018 alone (Agrawal et al., 2018; Arnold et al., 2018; Avgerinos et al., 2018; Benedict and Grillo, 2018; Bloom et al., 2018; Schmid et al., 2018; Vieira et al., 2018). With the limited number of available experimental reports only allowing preliminary, albeit reassuring conclusions on safety and side-effects of intranasal insulin (Schmid et al., 2018), it remains to be seen if the beneficial effects seen after acute or limited long-term intranasal insulin delivery will be corroborated, and can be put to use, in the clinical setting, or if exogenous insulin delivery implies the risk of "induced brain insulin resistance." Against this background, efficient lifestyle interventions are still a most desirable means to improve and maintain metabolic and cognitive

health (Stefan et al., 2016; Kivipelto et al., 2018), and their beneficial outcomes may very well rely on enhanced brain insulin signaling.

Acknowledgments

This work was by grants from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.; 01GI0925).

Conflict of interest

The authors declare no conflicts of interest.

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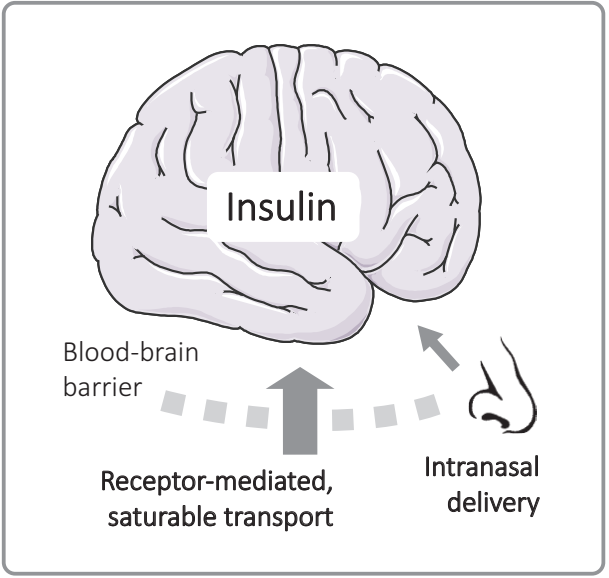
Figure 1. Schematic overview of insulin effects on and mediated by the CNS. Pancreatic insulin crosses the blood-brain barrier (BBB) to reach relevant sites of action ; intranasally administered insulin bypasses the BBB to exert respective functional effects as shortly summarized in the figure and discussed in detail in the text.



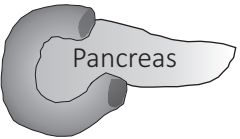
- *enhances declarative and working memory*
- *increases brain energy levels and functional connectivity*

- *decreases food intake and body weight*
- *decreases brain responses to food stimuli*

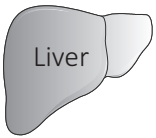
- *increases sleep-associated growth hormone release and slow oscillation power*

HPA

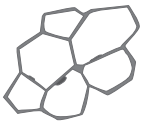
- *dampens stress-induced and sleep-associated hypothalamic-pituitary-adrenal axis activity*



- *increases pancreatic insulin secretion*



- *inhibits endogenous glucose production*



- *inhibits lipolysis and stimulates lipogenesis*

- *enhances postprandial thermogenesis*