Intranasal insulin as a therapeutic option in the treatment of cognitive impairments

Christian Benedict [a,b,*], William H. Frey II [c], Helgi B. Schiöth [a], Bernd Schultes [d],
Jan Born [b], Manfred Hallschmid [b]

[a] Department of Neuroscience, University of Uppsala, Sweden
[b] Department of Neuroendocrinology, University of Lübeck, Germany
[c] Alzheimer's Research Center at Regions Hospital, St. Paul, MN, USA
[d] Kantonsspital St. Gallen, Interdisciplinary Obesity Center, Switzerland

ARTICLE INFO

ABSTRACT

The brain is a major target of circulating insulin. Enhancing central nervous insulin action has been shown to improve memory functions in animals as well as in humans, benefitting in particular hippocampus-dependent (declarative) memory. As Alzheimer's disease (AD) is associated with reduced central nervous insulin signaling and attenuated permeation of blood-borne insulin across the blood-brain-barrier, the cognitive decline in AD patients may at least in part be derived from impaired brain insulin signaling. Thus, therapeutic strategies to overcome central nervous system insulin deficiency and resistance might be an attractive option in the treatment of cognitive impairments like AD. Insulin can be effectively delivered directly to the brain via the intranasal route that enables the hormone to bypass the blood-brain barrier and modulate central nervous functions. This review summarizes a series of studies demonstrating beneficial effects of intranasal insulin on memory functions both in healthy humans and in patients with cognitive impairments such as AD. These experiments in humans consistently indicate that enhancing brain insulin signaling by intranasal administration of the hormone improves hippocampus-dependent memory in the absence of adverse side effects. Considering that insulin also acts as a neuroprotective signal, up-regulating brain insulin levels by intranasal insulin administration appears to be a promising approach in the treatment and prevention of central nervous system insulin deficiency and resistance as found in AD.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

The successful isolation of insulin from the pancreas and the demonstration that the hormone effectively normalizes blood glucose levels in near-fatal cases of diabetes were milestones of modern medical science (Banting and Best, 1922). Because brain glucose metabolism appeared to be unaffected by insulin [for instance, see (Park and Johnson, 1955)], the central nervous system (CNS) for a long time was considered to be an insulin-independent organ. However, the discovery of insulin and its receptors in the CNS nearly six decades after the first purification of the hormone vitiated this assumption (Havrankova et al., 1978b). In the meantime, it has been shown in a number of different species including humans that enhanced brain insulin signaling decreases food intake and body weight, pointing to an important physiological role of the hormone in the CNS [(Woods et al., 1979; Hallschmid et al., 2004a), for review, see (Porte et al., 2005)]. Insulin, reaching the brain via a receptor-mediated, saturable transport system (Woods et al., 2003), along with leptin provides the CNS with negative feedback on body fat stores,

thus essentially contributing to the central nervous control of body weight. Also, high densities of insulin receptors are present in the hippocampus (Havrankova et al., 1978a), a structure that is highly relevant for the formation of declarative memory, i.e., the conscious retention and recollection of facts and events (Squire and Zola, 1996). The assumption that insulin thus may influence declarative memory functions was corroborated by reports showing that the direct administration of insulin into the CNS acutely improves hippocampus-dependent memory tested during a passive avoidance task in rats (Park et al., 2000) and that higher as compared to lower doses of i.v. insulin enhance declarative memory functions in healthy men (Kern et al., 2001).

Remarkably, patients suffering from Alzheimer's disease (AD) often display disturbed cerebral glucoregulation as indicated by the progressive reduction of brain glucose metabolism observed in positron emission tomography studies (Mosconi et al., 2009). They also show reduced brain insulin receptor activity along with lower cerebrospinal fluid (CSF) insulin levels and peripheral hyperinsuline-mia (Craft et al., 1998; Zhao and Townsend, 2009) and attenuated insulin and insulin-like growth factor expression (Steen et al., 2005), suggesting that impaired brain insulin signaling plays a critical role in the loss of memory functions associated with this disease. Against this background, raising CSF insulin concentrations may be expected to

* Corresponding author. Department of Neuroscience, Functional Pharmacology, Uppsala University, BMC, P.O. Box 593, 751 24 Uppsala, Sweden.
E-mail address: christian.benedict@neuro.uu.se (C. Benedict).

counteract AD related memory deficits. Supporting this assumption, enhancing brain insulin levels in AD patients by intravenous insulin administration has been shown to acutely improve performance on a verbal (i.e., hippocampus-dependent) memory task (Craft et al., 1996). However, owing to the strong peripheral side effects of intravenous insulin infusion, notably hypoglycemia, and the fact that high systemic doses would be needed to achieve functionally effective insulin concentrations in the CSF, this mode of administration is not viable in the clinical setting. In contrast, the intranasal route may be a promising approach enabling the selective elevation of central nervous insulin levels while avoiding adverse side effects (Born et al., 2002; Francis et al., 2009).

2. Intranasal delivery of substances to the brain

The blood-brain barrier (BBB) is a natural barrier protecting the brain from damage by harmful substances that might be ingested or otherwise enter the blood stream. Intranasal administration circumvents the BBB to rapidly and directly deliver therapeutics to the CNS. The olfactory region has unique anatomic and physiologic attributes that provide both extracellular and intracellular pathways into the CNS that bypass the BBB (Thorne et al., 1995; Illum, 2000; Hanson and Frey, 2008; Dhuria et al., 2010). Olfactory sensory neurons are the only first order neurons whose cell bodies are located in a distal epithelium. Their dendrites are directly exposed to the external environment of the upper nasal passage while their axons project through perforations in the cribriform plate of the ethmoid bone to synaptic glomeruli in the olfactory bulb. Previously, we have demonstrated that intranasal administration of insulin provides direct access of the hormone to the CSF within 30 min without substantial uptake into the bloodstream (Born et al., 2002). Given that intraneuronal, axonal transport requires hours to days for substances to reach the brain (Thorne et al., 1995), it is more plausible to assume that intranasal insulin, passing through intercellular clefts in the olfactory epithelium, diffuses into the subarachnoidal space and is rapidly delivered to CSF (Born et al., 2002) and brain tissue (Francis et al., 2008, 2009) via bulk flow transport (Thorne and Frey, 2001) through perineural channels (Dhuria et al., 2010). In addition to the olfactory pathway, insulin, insulin-like growth factor-I and other therapeutics have also been shown to be rapidly transported from the nasal mucosa to the brain along the trigeminal neural pathway (Francis et al., 2008; Ross et al., 2008; Thorne et al., 2008, 2004).

Experiments in humans have shown that intranasal insulin exerts rapid effects on EEG parameters (Hallschmid et al., 2004b). Such changes are roughly comparable to those induced by intravenous bolus injections of the hormone, indicating that following intranasal administration, a significant amount of the applied dose reaches the brain in a functionally active state (Hallschmid et al., 2004b).
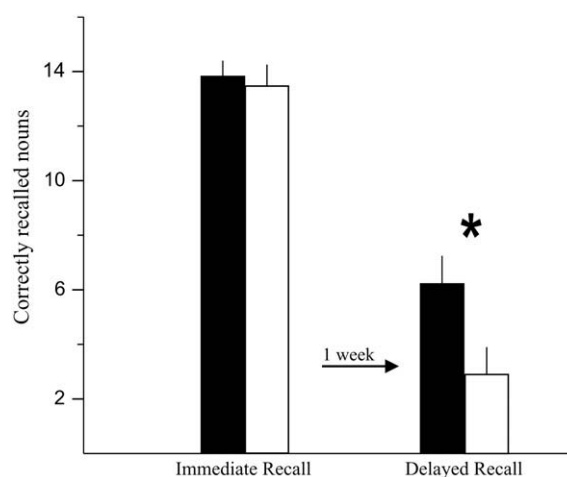
3. Intranasal insulin improves memory in healthy humans

Intranasal administration of insulin has repeatedly been demonstrated to improve memory functions relying on the hippocampus that displays a high density of insulin receptors (Havrankova et al., 1978b). In a human study (Benedict et al., 2004), we assessed the effects of intranasal insulin on memory performance in 38 healthy young volunteers who were intranasally administered either placebo or insulin (160 IU/d) during an eight-week treatment phase. At the beginning and end of treatment, lists of 30 nouns (e.g., tree, father, and chocolate) were orally presented to the subjects. In addition to an immediate recall 3 min after presentation of the words, in a delayed recall session taking place one week later subjects wrote down all words they still remembered. Whereas immediate recall performance was unaffected by seven weeks of pretreatment with intranasal insulin, subjects of the insulin as compared to the placebo group remembered significantly more words one week after the presenta-

tion (Fig. 1). In a further study (Benedict et al., 2007), we focused on the effects of intranasally administered insulin aspart on hippocampus-dependent memory, applying the same study design as described above (Benedict et al., 2004). After subcutaneous administration, insulin aspart is more rapidly absorbed than regular human insulin (Kang et al., 1991) due to faster dissociation from hexamers into monomers and dimers (Brange and Volund, 1999). Accordingly, we hypothesized that insulin aspart would access the brain compartment with higher efficiency than regular insulin and exert stronger effects on memory function. After 8 weeks of treatment, delayed word list recall as compared to placebo treatment was significantly improved in both the insulin aspart and regular human insulin groups. In line with our hypothesis, insulin aspart-treated subjects performed even better than those of the regular human insulin group. Recent data indicate that the memory-improving effects of acutely administered insulin are more pronounced in women than in men, pointing to the potential relevance of gender differences in the emergence of central nervous insulin effects (Benedict et al., 2008).

4. Intranasal insulin improves memory function in AD patients

Based on clinical evidence linking AD with disturbed brain insulin signaling, Craft and colleagues assessed the acute effects of different doses of intranasal insulin (10, 20, 40, 60 IU) on hippocampus-dependent memory function in memory-impaired subjects (early stage AD or amnestic mild cognitive impairment − MCI) in comparison to age-matched healthy controls (Reger et al., 2006, 2008a). After receiving a nasal dose of insulin or placebo, subjects were presented a list of words and were asked to recall as many items as possible. Intranasal insulin treatment produced significant memory improvement, with the patients showing maximal benefits when 20 IU were administered. In both studies, memory-improving effects of intranasal insulin were found only in non-carriers of the APOE4 gene allele that is linked to an increased risk of developing AD (Cummings and Cole, 2002), whereas the APOE4-postive subjects showed no benefits or even a decline in memory function. It is currently unclear whether this difference is due to the stronger association between insulin resistance and AD found in patients without as compared to those with the risk allele (Craft et al., 1998) or if insulin administration may aggravate impairments in central
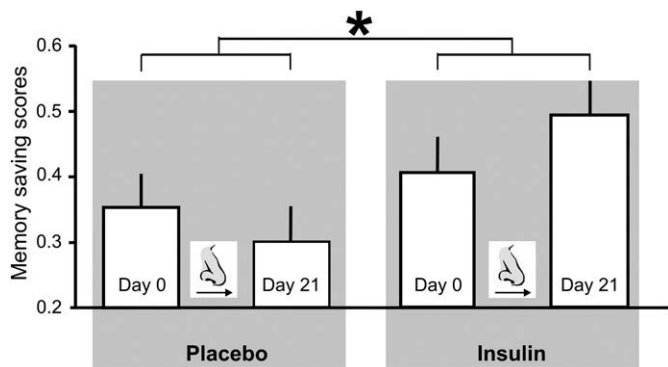

Fig. 1. Intranasal insulin improves memory in humans [data from (Benedict et al., 2004)]. Immediate and delayed word list recall in healthy humans following 7 (immediate recall) and 8 weeks (delayed recall) of intranasal administration of regular human insulin (160 IU/day, black bars, $n = 19$) and placebo (white bars, $n = 19$), respectively. Immediate recall was tested 3 min after the auditory presentation of 30 nouns (e.g., car, tree, and chocolate). Delayed recall of the same list of nouns was tested after one more week of treatment. Baseline adjusted means ± SEM are indicated. *$P \leq 0.05$, for pairwise comparisons between groups.

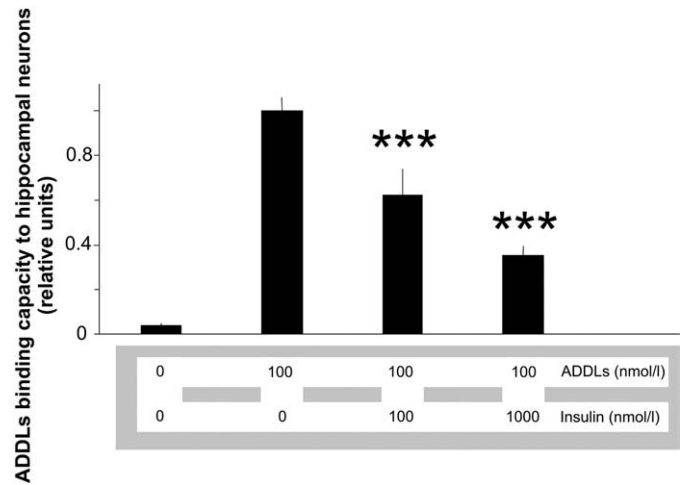nervous glucose metabolism present in carriers of the APOE4-positive genotype (Reiman et al., 2004).

In order to further examine the therapeutic potential of intranasal insulin in individuals with early stage AD or amnestic MCI, Craft and coworkers treated patients with daily insulin doses for three weeks and tested their memory performance before and after (Reger et al., 2008b). In each session, subjects listened to a story containing 44 informational bits and were then asked to recall the story immediately and after a 20-minute delay. Memory savings were calculated as the relative amount of information retained over the delay between immediate and delayed recall. Relative to baseline performance, at day 21 insulin-treated AD and MCI subjects displayed greater memory savings than placebo-assigned subjects (Fig. 2). Taken together, the results gained in healthy and memory-impaired humans indicate a beneficial memory effect of intranasal insulin preferentially with regard to hippocampus-dependent declarative memory functions. Given that cognitive impairments and diabetes are hypothesized to share the pathogenic feature of central nervous insulin resistance, it is important to note in this context that in a rodent model of diabetes, intranasal insulin prevented cognitive decline, cerebral atrophy and white matter changes in the brain (Francis et al., 2008).

5. Potential mechanisms of insulin-induced memory improvements

Several mechanisms may contribute to the memory-improving effect of central nervous insulin signaling. Experimental findings link insulinergic input to regional improvements of glucose metabolism (Bingham et al., 2002), enhanced synaptic long-term potentiation (Lee et al., 2009), modulations of the action of neurotransmitters like norepinephrine and acetylcholine (Gerozissis, 2003) and attenuated cortisol secretion (Benedict et al., 2004; Hallschmid et al., 2008). Insulin in the brain also stimulates the formation of an insulin degrading enzyme that is capable of degrading beta amyloid (Aβ), whose strong accumulation in the brains of AD patients is assumed to be a pathogenic hallmark of the disease (Craft, 2009). Small oligomers of the peptide, known as Aβ-derived diffusible ligands (ADDLs), are potent CNS neurotoxins (Lambert et al., 1998). They attach to neuronal synapses with high specificity, thereby acting as pathogenic ligands (Gong et al., 2003). In a recent report, insulin was shown to significantly mitigate pathological binding of ADDLs to synapses of hippocampal neurons [Fig. 3; (De Felice et al., 2009)]. Moreover, insulin has been reported to regulate glycogen-synthase kinase-3-beta, thereby inhibiting the phosphorylation of tau protein that forms neurofibrillary tangles which are a further pathological feature of AD (Hong and Lee, 1997). Insulin has also been shown to maintain synaptic density (Chiu et al., 2008) and to reduce the hypothalamic–

Fig. 3. Insulin blocks the binding of neuronal Aβ-derived diffusible ligands (ADDLs) to hippocampal neuronal cultures [modified with permission from (De Felice et al., 2009)]. Insulin (100 nM or 1 μM) was added 30 min before ADDLs were administered. Binding of ADDLs to hippocampal neurons was detected using NU4 antibody. Significant differences to non-insulin-treated ADDL-treated cultures are indicated (***$P \leq 0.007$).

pituitary–adrenal axis response to stress that can have deleterious effects on the hippocampus (Bohringer et al., 2008).

6. Concluding remarks

The studies briefly summarized here support the assumption that brain insulin signaling essentially contributes to memory function in humans. Accordingly, experimental findings suggest that central nervous system insulin deficiency and resistance is a pathophysiologic core feature of cognitive impairments like AD (Zhao and Townsend, 2009; Craft, 2009), even prompting some clinical researchers to refer to this disease as "Type 3 diabetes" (de la Monte and Wands, 2008). Enhancing insulin signaling in the brain by means of intranasal insulin administration may therefore be a useful option in the treatment and possibly even the prevention of this devastating disease.

Fig. 2. Intranasal insulin improves memory in early stage AD [modified with permission from (Reger et al., 2008b)]. Mean memory saving scores (±SEM) in patients with early stage AD at baseline (day 0) and after 21 days of treatment with placebo ($n = 12$) and insulin ($n = 13$), respectively. Insulin-treated patients showed increased memory savings over the 21-day period relative to placebo (*$P \leq 0.05$).

References

Banting, F., Best, C., 1922. Insulin its chemistry and physiology. J. Lab. Clin. Med. 7, 251–266.

Benedict, C., Hallschmid, M., Hatke, A., Schultes, B., Fehm, H.L., Born, J., Kern, W., 2004. Intranasal insulin improves memory in humans. Psychoneuroendocrinology 29 (10), 1326–1334.

Benedict, C., Hallschmid, M., Schmitz, K., Schultes, B., Ratter, F., Fehm, H.L., Born, J., Kern, W., 2007. Intranasal insulin improves memory in humans: superiority of insulin aspart. Neuropsychopharmacology 32 (1), 239–243.

Benedict, C., Kern, W., Schultes, B., Born, J., Hallschmid, M., 2008. Differential sensitivity of men and women to anorexigenic and memory improving effects of intranasal insulin. J. Clin. Endocrinol. Metab. 93 (4), 1339–1344.

Bingham, E.M., Hopkins, D., Smith, D., Pernet, A., Hallett, W., Reed, L., Marsden, P.K., Amiel, S.A., 2002. The role of insulin in human brain glucose metabolism: an 18fluoro-deoxyglucose positron emission tomography study. Diabetes 51 (12), 3384–3390.

Bohringer, A., Schwabe, L., Richter, S., Schachinger, H., 2008. Intranasal insulin attenuates the hypothalamic–pituitary–adrenal axis response to psychosocial stress. Psychoneuroendocrinology 33 (10), 1394–1400.

Born, J., Lange, T., Kern, W., McGregor, G.P., Bickel, U., Fehm, H.L., 2002. Sniffing neuropeptides: a transnasal approach to the human brain. Nat. Neurosci. 5 (6), 514–516.

Brange, J., Volund, A., 1999. Insulin analogs with improved pharmacokinetic profiles. Adv. Drug Deliv. Rev. 35 (2–3), 307–335.

Chiu, S.L., Chen, C.M., Cline, H.T., 2008. Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo. Neuron 58 (5), 708–719.

Craft, S., 2009. The role of metabolic disorders in Alzheimer disease and vascular dementia: two roads converged. Arch. Neurol. 66 (3), 300–305.

Craft, S., Newcomer, J., Kanne, S., Gogo-Jack, S., Cryer, P., Sheline, Y., Luby, J., Gogo-Jack, A., Alderson, A., 1996. Memory improvement following induced hyperinsulinemia in Alzheimer's disease. Neurobiol. Aging 17 (1), 123–130.

Craft, S., Peskind, E., Schwartz, M.W., Schellenberg, G.D., Raskind, M., Porte Jr., D., 1998. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. Neurology 50 (1), 164–168.

Cummings, J.L., Cole, G., 2002. Alzheimer disease. JAMA 287 (18), 2335–2338.

De Felice, F.G., Vieira, M.N., Bomfim, T.R., Decker, H., Velasco, P.T., Lambert, M.P., Viola, K.L., Zhao, W.Q., Ferreira, S.T., Klein, W.L., 2009. Protection of synapses against Alzheimer's-linked toxins: insulin signaling prevents the pathogenic binding of Abeta oligomers. Proc. Natl Acad. Sci. USA 106 (6), 1971–1976.

de la Monte, S.M., Wands, J.R., 2008. Alzheimer's disease is type 3 diabetes-evidence reviewed. J. Diabetes Sci. Technol. 2 (6), 1101–1113.

Dhuria, S.V., Hanson, L.R., Frey, W.H., 2010. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J. Pharm. Sci. 99 (4), 1654–1673.

Francis, G.J., Martinez, J.A., Liu, W.Q., Xu, K., Ayer, A., Fine, J., Tuor, U.I., Glazner, G., Hanson, L.R., Frey, W.H., Toth, C., 2008. Intranasal insulin prevents cognitive decline, cerebral atrophy and white matter changes in murine type I diabetic encephalopathy. Brain 131 (Pt 12), 3311–3334.

Francis, G., Martinez, J., Liu, W., Nguyen, T., Ayer, A., Fine, J., Zochodne, D., Hanson, L.R., Frey, W.H., Toth, C., 2009. Intranasal insulin ameliorates experimental diabetic neuropathy. Diabetes 58 (4), 934–945.

Gerozissis, K., 2003. Brain insulin: regulation, mechanisms of action and functions. Cell. Mol. Neurobiol. 23 (1), 1–25.

Gong, Y., Chang, L., Viola, K.L., Lacor, P.N., Lambert, M.P., Finch, C.E., Krafft, G.A., Klein, W.L., 2003. Alzheimer's disease-affected brain: presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. Proc. Natl Acad. Sci. USA 100 (18), 10417–10422.

Hallschmid, M., Benedict, C., Schultes, B., Fehm, H.L., Born, J., Kern, W., 2004a. Intranasal insulin reduces body fat in men but not in women. Diabetes 53 (11), 3024–3029.

Hallschmid, M., Schultes, B., Marshall, L., Molle, M., Kern, W., Bredthauer, J., Fehm, H.L., Born, J., 2004b. Transcortical direct current potential shift reflects immediate signaling of systemic insulin to the human brain. Diabetes 53 (9), 2202–2208.

Hallschmid, M., Benedict, C., Schultes, B., Born, J., Kern, W., 2008. Obese men respond to cognitive but not to catabolic brain insulin signaling. Int. J. Obes. (Lond.) 32 (2), 275–282.

Hanson, L.R., Frey, W.H., 2008. Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. BMC Neurosci. 9 (Suppl 3) S5-.

Havrankova, J., Roth, J., Brownstein, M., 1978a. Insulin receptors are widely distributed in the central nervous system of the rat. Nature 272 (5656), 827–829.

Havrankova, J., Schmechel, D., Roth, J., Brownstein, M., 1978b. Identification of insulin in rat brain. Proc. Natl. Acad. Sci. U. S. A. 75 (11), 5737–5741.

Hong, M., Lee, V.M., 1997. Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. J. Biol. Chem. 272 (31), 19547–19553.

Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11 (1), 1–18.

Kang, S., Creagh, F.M., Peters, J.R., Brange, J., Volund, A., Owens, D.R., 1991. Comparison of subcutaneous soluble human insulin and insulin analogues (AspB9, GluB27; AspB10; AspB28) on meal-related plasma glucose excursions in type I diabetic subjects. Diab. Care 14 (7), 571–577.

Kern, W., Peters, A., Fruehwald-Schultes, B., Deininger, E., Born, J., Fehm, H.L., 2001. Improving influence of insulin on cognitive functions in humans. Neuroendocrinology 74 (4), 270–280.

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A., Klein, W.L., 1998. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. U. S. A. 95 (11), 6448–6453.

Lee, C.C., Kuo, Y.M., Huang, C.C., Hsu, K.S., 2009. Insulin rescues amyloid beta-induced impairment of hippocampal long-term potentiation. Neurobiol. Aging 30 (3), 377–387.

Mosconi, L., Mistur, R., Switalski, R., Tsui, W.H., Glodzik, L., Li, Y., Pirraglia, E., De, S.S., Reisberg, B., Wisniewski, T., de Leon, M.J., 2009. FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease. Eur. J. Nucl. Med. Mol. Imaging 36 (5), 811–822.

Park, C.R., Johnson, L.H., 1955. Effect of insulin on transport of glucose and galactose into cells of rat muscle and brain. Am. J. Physiol. 182 (1), 17–23.

Park, C.R., Seeley, R.J., Craft, S., Woods, S.C., 2000. Intracerebroventricular insulin enhances memory in a passive-avoidance task. Physiol. Behav. 68 (4), 509–514.

Porte Jr., D., Baskin, D.G., Schwartz, M.W., 2005. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. Diabetes 54 (5), 1264–1276.

Reger, M.A., Watson, G.S., Frey, W.H., Baker, L.D., Cholerton, B., Keeling, M.L., Belongia, D.A., Fishel, M.A., Plymate, S.R., Schellenberg, G.D., Cherrier, M.M., Craft, S., 2006. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. Neurobiol. Aging 27 (3), 451–458.

Reger, M.A., Watson, G.S., Green, P.S., Baker, L.D., Cholerton, B., Fishel, M.A., Plymate, S.R., Cherrier, M.M., Schellenberg, G.D., Frey, W.H., Craft, S., 2008a. Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. J. Alzheimers Dis. 13 (3), 323–331.

Reger, M.A., Watson, G.S., Green, P.S., Wilkinson, C.W., Baker, L.D., Cholerton, B., Fishel, M.A., Plymate, S.R., Breitner, J.C., Degroodt, W., Mehta, P., Craft, S., 2008b. Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology 70 (6), 440–448.

Reiman, E.M., Chen, K., Alexander, G.E., Caselli, R.J., Bandy, D., Osborne, D., Saunders, A.M., Hardy, J., 2004. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc. Natl. Acad. Sci. U. S. A. 101 (1), 284–289.

Ross, T.M., Zuckermann, R.N., Reinhard, C., Frey, W.H., 2008. Intranasal administration delivers peptoids to the rat central nervous system. Neurosci. Lett. 439 (1), 30–33.

Squire, L.R., Zola, S.M., 1996. Structure and function of declarative and nondeclarative memory systems. Proc. Natl. Acad. Sci. U. S. A. 93 (24), 13515–13522.

Steen, E., Terry, B.M., Rivera, E.J., Cannon, J.L., Neely, T.R., Tavares, R., Xu, X.J., Wands, J.R., de la Monte, S.M., 2005. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J. Alzheimers Dis. 7 (1), 63–80.

Thorne, R.G., Frey, W.H., 2001. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. Clin. Pharmacokinet. 40 (12), 907–946.

Thorne, R.G., Emory, C.R., Ala, T.A., Frey, W.H., 1995. Quantitative analysis of the olfactory pathway for drug delivery to the brain. Brain Res. 692 (1–2), 278–282.

Thorne, R.G., Pronk, G.J., Padmanabhan, V., Frey, W.H., 2004. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 127 (2), 481–496.

Thorne, R.G., Hanson, L.R., Ross, T.M., Tung, D., Frey, W.H., 2008. Delivery of interferon-beta to the monkey nervous system following intranasal administration. Neuroscience 152 (3), 785–797.

Woods, S.C., Lotter, E.C., McKay, L.D., Porte Jr., D., 1979. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 282 (5738), 503–505.

Woods, S.C., Seeley, R.J., Baskin, D.G., Schwartz, M.W., 2003. Insulin and the blood-brain barrier. Curr. Pharm. Des. 9 (10), 795–800.

Zhao, W.Q., Townsend, M., 2009. Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease. Biochim. Biophys. Acta 1792 (5), 482–496.