Published in final edited form as:

Neurobiol Learn Mem. 2010 May ; 93(4): 546–553. doi:10.1016/j.nlm.2010.02.002.

Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance

Ewan C. McNay^{a,b,*}, Cecilia T. Ong^b, Rory J. McCrimmon^b, James Cresswell^b, Jonathan S. Bogan^b, and Robert S Sherwin^b

^a University at Albany, Albany, NY 12222, USA

^b Yale University School of Medicine, New Haven, CT 06520-8020, USA

Abstract

Insulin regulates glucose uptake and storage in peripheral tissues, and has been shown to act within the hypothalamus to acutely regulate food intake and metabolism. The machinery for transduction of insulin signaling is also present in other brain areas, particularly in the hippocampus, but a physiological role for brain insulin outside the hypothalamus has not been established. Recent studies suggest that insulin may be able to modulate cognitive functions including memory. Here we report that local delivery of insulin to the rat hippocampus enhances spatial memory, in a PI-3-kinase dependent manner, and that intrahippocampal insulin also increases local glycolytic metabolism. Selective blockade of endogenous intrahippocampal insulin signaling impairs memory performance. Further, a rodent model of type 2 diabetes mellitus produced by a high-fat diet impairs basal cognitive function and attenuates both cognitive and metabolic responses to hippocampal insulin administration. Our data demonstrate that insulin is required for optimal hippocampal memory processing. Insulin resistance within the telencephalon may underlie the cognitive deficits commonly reported to accompany type 2 diabetes.

Keywords

Glucose; Insulin; Spatial memory; Hippocampus; Diabetes

1. Introduction

Since its first identification and characterization in 1922 (Banting & Best, 1922), insulin has been established as the primary hormonal regulator of systemic glucose concentration. Insulin acts both to suppress hepatic glucose output and to stimulate glucose uptake in muscle and adipose tissues. The latter effect occurs via activation of the phosphatidylinositol-3-kinase (PI3K) signaling pathway and subsequent translocation of the insulin-responsive glucose transporter GluT4 to the cell surface. More recently, it has been shown that insulin can also modulate feeding behavior and metabolism via actions within the hypothalamus (and in particular within the arcuate nucleus (Plum, Belgardt, & Bruning, 2006; Schulingkamp, Pagano, Hung, & Raffa, 2000; Schwartz, Figlewicz, Baskin, Woods, & Porte, 2000; Woods, Schwartz, Baskin, & Seeley, 2000)). The remainder of the brain has generally been considered to be an insulin-insensitive organ, largely based on the fact that whole-brain glucose uptake and metabolism measurements have consistently shown little effect of insulin on total cerebral glucose metabolism, in line with some recent MRS and PET studies of the cerebral cortex

* Corresponding author. Address: Dept. of Psychology, University at Albany, SS369, 1400 Washington Avenue, Albany, NY 12222, USA. Fax: +1 518 442 4867. emcnay@albany.edu (E.C. McNay)..

(Hasselbalch et al., 1999; Seaquist, Damberg, Tkac, & Gruetter, 2001). However, recent studies have suggested that the hippocampus, which expresses high levels of insulin receptors (Dore, Kar, Rowe, & Quirion, 1997), as well as GluT4 (McEwen & Reagan, 2004; Reagan, 2005; Vannucci et al., 1998) and PI3K (Figlewicz & Szot, 1991), might be responsive to insulin. Insulin has been reported to cause translocation of GluT 4 to the membrane of hippocampal neurons in similar fashion to its effects in the periphery (McEwen & Reagan, 2004; Reagan, 2005), suggesting that insulin might increase hippocampal glucose uptake and/or metabolism, in contrast to the findings when the brain is taken as a whole.

There is an extensive literature showing that hippocampal cognitive performance is critically dependent on an adequate supply of glucose to meet the local metabolic demands of cognitive processes, and that provision of additional glucose to the hippocampus can enhance memory performance (Gold, 2005; McNay, Fries, & Gold, 2000; McNay & Gold, 2002). Furthermore, *in vitro* studies have shown that insulin can modulate hippocampal synaptic plasticity (Izumi, Yamada, Matsukawa, & Zorumski, 2003; van der Heide, Kamal, Artola, Gispen, & Ramakers, 2005; Zhao, Chen, Quon, & Alkon, 2004), i.e.v. administration of insulin has been shown to enhance performance on a passive-avoidance memory task (Park, Seeley, Craft, & Woods, 2000), and spatial memory training has been reported to alter hippocampal expression of insulin receptors (Zhao et al., 1999; Zhao et al., 2004). Hence, there are substantial reasons for believing that insulin might act within the hippocampus, a key brain area for learning and memory, to support memory performance. In keeping with this possibility, recent clinical observations in Alzheimer's disease (AD) patients suggest that insulin may enhance performance on hippocampally-mediated tasks (Watson & Craft, 2004), and enhancement of memory following systemic glucose administration has been suggested to be mediated, at least in part, via an increase in insulin delivery to the brain (Craft & Watson, 2004; Craft et al., 1996; Kern et al., 2001; Long, Davis, Garofalo, Spangler, & Ingram, 1992). Consistent with this latter suggestion, recent animal studies have suggested that delivery of insulin to the hippocampus might be able to modulate hippocampal memory processes. Unfortunately these studies have to date used both hyperphysiological insulin doses and aversive paradigms with elevated systemic glucose and epinephrine, making interpretation of any insulin effect difficult (Babri, Badie, Khamenei, & Seyedlar, 2007; Moosavi, Naghdi, & Choopani, 2007; Moosavi, Naghdi, Maghsoudi, & Asl, 2006).

A potential role for insulin in enhancement of cognitive function is of particular interest in the context of type 2 diabetes mellitus (T2DM). T2DM is characterized by systemic hyperglycemia and insulin resistance and is associated with deficits in cognitive functions, including memory (Gispen & Biessels, 2000; Winocur et al., 2005). Animal models of T2DM show impaired hippocampal translocation of GluT4 (Reagan, 2005; Winocur et al., 2005), reduced hippocampal synaptic plasticity (Mielke et al., 2005), and attenuated hypothalamic responsiveness to insulin in control of food intake (Clegg et al., 2005; Figlewicz et al., 2004) as well as reduced temporal lobe insulin signaling (Moroz, Tong, Longato, Xu, & Monte, 2008). Moreover, neuronal insulin resistance has been suggested to be directly linked to the development of neurodegenerative disease (Schubert et al., 2004), and drugs which improve sensitivity to insulin are currently being tested in human trials for treatment of AD; indeed, AD has been described as "Type 3 diabetes" (Steen et al., 2005). Here, we show that acute delivery of physiological doses of insulin, but not IGF-1, directly to the hippocampus specifically enhances spatial working memory via a PI3K-dependent mechanism, and increases local glucose removal from interstitial fluid, whereas blockade of endogenous hippocampal insulin either with PI-3-kinase antagonists or small anti-insulin antibody-like peptides impairs cognitive performance below baseline. Diet-induced as before, T2DM reduces baseline hippocampally-mediated cognitive function and impairs the ability of insulin to enhance memory performance and/or increase hippocampal fuel metabolism. Importantly, we also show that direct, specific blockade of *endogenous* intrahippocampal insulin produces marked

cognitive deficits, supporting a role for insulin in physiological hippocampal memory processes.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA), were studied, starting at one month of age. Rats were individually housed, with food and water available *ad libitum*, on a 12:12 h light:dark schedule (lights on 0700 h). At 6 weeks of age, animals were randomly assigned to either standard lab chow (control animals, 6% fat, Purina) or a high-fat (diet-induced obese (DIO) and diet-resistant (DR) animals, 31.8% fat by calories from butter and corn oil, Research Diets #D12266B) chow and maintained on this diet until cognitive testing at 20 weeks. Room/cage temperature was maintained at 70 F. All procedures were approved by the Yale University Institutional Animal Care and Use Committee. Animals were handled regularly prior to testing. At the time of testing, the top tertile of high-fat diet animals, by weight gain, was defined as DIO; the bottom tertile was defined as DR.

2.2. Surgery

Animals received either a microinjection guide cannula (Plastics One) or microdialysis guide cannula (CMA12, CMA/Microdialysis) into the left hippocampus as described previously (McNay et al., 2000) with coordinates relative to bregma +3.8 mm AP, +5.0 mm lateral, –4.5 mm from dura, nosebar at 5.0 mm above interaural line. Animals were given 1 week for recovery post-surgery prior to testing during which time they were handled extensively daily.

2.3. Sample analysis

Microdialysis samples were assayed for glucose and lactate using a CMA600 analyzer. Measurements were corrected for *in vivo* probe recovery using the slope of a hippocampal ECF zero-net-flux plot under the same experimental conditions (McNay & Sherwin, 2004b; McNay et al., 2000). Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Catecholamine analysis was performed by high-performance liquid chromatography (ESA, Acton, MA). Insulin analysis was done by radioimmunoassay (Linco Research).

2.4. Microinjection/microdialysis procedures

Microinjections were given to maze-tested animals over 2 min in a total volume of 0.5 μ L, into the left hippocampus, 10 min prior to testing. The vehicle for all groups (including controls) was artificial ECF (aECF; 153.5 mM Na, 4.3 mM K, 0.41 mM Mg, 0.71 mM Ca, 139.4 mM Cl, 1.25 mM glucose, buffered at pH 7.4 (McNay & Sherwin, 2004b)) with the exception of groups receiving Affibodies, which were administered as supplied in PBS. Injection cannulae were left in place for a further 2 min after injection to ensure diffusion. Animals used for microdialysis measurements had a fresh probe inserted into the left hippocampus on the morning of measurement, followed by an acclimation period of at least 2 h. The dialysis membrane was 3 mm long and thus sampled across several regions of the hippocampus. Rats were allowed to move freely in their home cages throughout measurement. Insulin was administered via reverse dialysis, a liquid switch allowing addition to the microdialysis perfusate without disturbance to the animal. Probes were perfused at 1.5 μ L/min with artificial extracellular fluid as previously described (McNay et al., 2000). All reagents were obtained from Sigma (St. Louis, MO).

2.5. Alternation memory testing

Rats were placed into the center of a four-arm maze and allowed to explore for 20 min. Rats spontaneously alternate between unbaited maze arms, using spatial working memory to retain knowledge of arms previously visited. Spontaneous alternation has been extensively used as a spatial working memory task that examines acute learning and memory (e.g. Conrad, Lupien, & McEwen, 1999; Dember, 1989; McNay & Gold, 1998; McNay et al., 2000; Sarter, Bodewitz, & Steckler, 1989; Winocur & Gagnon, 1998). Specifically, the measure of memory performance used was percent 4/5 alternation. An alternation is counted when the rat visits all four arms within any span of five consecutive arm choices; the maximum number of alternations is $N-4$, where N is the total number of arms entered. The actual number of alternations made is expressed as a percentage of this number. Chance level on this measure is 44%.

2.6. Euthanasia and histology

After maze testing, rats were killed by i.p. overdose of sodium pentobarbital, brains were removed, and sections were taken for confirmation of probe placement using cresyl violet staining for identification of probe track location; only data from animals with correct probe placements were included in analyses. Plasma samples for glucose and insulin measurements were taken from trunk blood following euthanasia and decapitation.

2.7. Phospho-Akt immunoblotting

Samples were obtained by punch biopsy, and were transferred immediately to 1× LDS NuPAGE sample buffer (Invitrogen Novex). After treatment with a handheld tissue grinder (Kontes), samples were heated to 95 °C for 10 min. in the presence of 2% 2-mercaptoethanol. Insoluble debris was pelleted by centrifugation at 13,200 rpm for 5 min. in an Eppendorf 5415C benchtop microfuge at room temperature. The supernatants were transferred to new tubes and placed on ice. Protein concentrations were measured in triplicate using an EZ-Q protein assay (Invitrogen Molecular Probes), which was read using a PerkinElmer Victor³ plate reader. Equal amounts (~12 µg) of each sample were separated on precast 4–12% gradient bis-Tris NuPAGE gels (Invitrogen), then transferred to nitrocellulose membranes using a semidry electrotransfer apparatus (Biorad). All gels were transferred simultaneously, immunoblotted in the same solutions, and exposed to film in parallel, so that conditions were identical and samples can be compared meaningfully. Immunoblotting was done as previously described (Yu, Cresswell, Loffler, & Bogan, 2007). Membranes were blocked for 1 h at room temperature in phosphate buffered saline containing 0.02% Tween-20 and 5% (w/v) nonfat dry milk. Primary antibodies were diluted 1:1000 in this same buffer, with the exception of the phospho-Akt antibody which was used at 1:500 in phosphate buffered saline, 0.02% Tween-20, 5% bovine serum albumin. Antibodies used were directed to phospho-Akt-Ser473 (Cell Signaling Technology Cat. No. 4060), Akt (pan-isoform, Cell Signaling Cat. No. 4691, and Hsc70 (Stressgen Bioreagents, Cat. No. SPA-815). Peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch, and were used at 1:50,000 in phosphate buffered saline containing 0.02% Tween-20 and 5% (w/v) nonfat dry milk. Signals were detected on film using enhanced chemiluminescence. Exposures in the linear range of the film were analyzed by densitometry. Films were imaged by transillumination on an Epson Expression 1680 scanner driven by SilverFast Ai software (Laser-soft Imaging, used as an Adobe Photoshop plugin). Images were acquired at 16 bit pixel depth, and linear gamma was maintained throughout. Quantification was done using ImageJ (NIH), and local background was subtracted for each band.

To meaningfully compare band intensities, all samples were electrophoresed, transferred, immunoblotted, and exposed to film in parallel, and insulin stimulated and control samples were run on the same gels. The mean and standard error of the band intensities for each group of samples was calculated and plotted using Excel (Microsoft) and Kaleidagraph (Synergy

Software). Statistical significance was assessed using a one-way ANOVA and Prism software (Graphpad). Because the main point of the experiment was to compare the insulin-stimulated ipsilateral samples to each of the other groups, Dunnett's multiple comparison test was used *post hoc* with an alpha value of 0.05 (95% confidence intervals).

In addition to comparing the mean intensities of the phospho- and total Akt bands from each group of samples, ratios of the band intensities were compared using Hsc70 as a loading control. Thus, both phospho-Akt and total Akt immunoblots were reprobbed using an antibody to Hsc70, as described above. This abundant 70 kD heat shock protein runs slightly above the Akt band (55 kD), and was detected using a rat antibody. Thus, Akt (detected using rabbit antibodies) was not also observed on the reprobbed membranes. By comparing P-Akt and total Akt each to Hsc70, detected on the same membrane, the need to strip the immunoblots was obviated (this can be a source of error). Importantly, calculation of the compound ratio, (P-Akt/Hsc70)/(total Akt/Hsc70), for each sample confirmed results using the mean band intensities within each group. Statistical analysis (one-way ANOVA followed by Dunnett's Multiple Range test) showed that this compound ratio was significantly greater for insulin-stimulated ipsilateral samples compared to all other groups of samples.

2.8. Statistical analysis

All tests were conducted using two-tailed, unpaired *t*-tests or one-way analysis of variance (ANOVA) with individual group differences vs. control determined by Dunnett's Multiple Comparison *post hoc* tests where indicated, with alpha = 0.05 in all cases. *N* of each behavioral group was between 8 and 14.

3. Results

3.1. Spatial working memory performance is specifically enhanced by intrahippocampal insulin administration

Spatial working memory is sensitive to acute manipulations of activity and metabolism within the left hippocampus (McNay & Gold, 1998; McNay, McCarty, & Gold, 2001; McNay et al., 2000). Administration of insulin (Humulin, Lilly) to the left hippocampus, 10 min prior to testing, produced an inverted-U dose-related enhancement of performance on the spontaneous alternation spatial memory task (Fig. 1). Doses of 100 μ U and 1 mU insulin significantly improved alternation performance, with both higher and lower doses having no performance effect. Administration of insulin to the striatum, a brain area involved in both motor function and some forms of memory, did not affect alternation performance (Fig. 2a). Insulin is able to bind to and activate the insulin-like growth factor 1 receptor (IGF-1R), albeit with much lower affinity than for the insulin receptor (Rechler & Nissley, 1985). To confirm that insulin was not acting to enhance memory via actions at hippocampal IGF-1R, we delivered IGF-1 to the left hippocampus at a dose either of equal protein concentration to that of the 100 μ U insulin group (to control for any effect of protein delivery *per se*), or of 1% of that concentration to match the activation of IGF-1R expected with 100 μ U insulin. No effect on memory performance was seen at either dose (Fig. 2a).

3.2. Insulin modulation of memory is via the PI3K cascade, not via binding to K_{ATP} channels

Both in the hypothalamus and peripherally, insulin's actions are commonly mediated by the PI3K signaling cascade (Niswender et al., 2003). To confirm that this pathway is also involved in insulin's modulation of hippocampal function, we coadministered the PI3K blockers wortmannin or LY294002 with 100 μ U insulin. Both drugs reversed the memory enhancement seen with insulin alone (Fig. 2b). These data suggest that insulin's ability to enhance memory is dependent on the activation of PI3K. Insulin is also known to open ATP-gated potassium channels (K_{ATP} ; (Spanswick, Smith, Mirshamshi, Routh, & Ashford, 2000)), including within

the hypothalamus (Pocai et al., 2005), and manipulations of hippocampal K_{ATP} have been shown to modulate spatial working memory (Stefani & Gold, 2001). To determine whether insulin might be acting at K_{ATP} to modulate memory, we delivered the specific K_{ATP} channel opener diazoxide at a range of doses prior to testing (Fig. 2c). However, diazoxide in fact produced a dose-dependent impairment of memory performance.

3.3. Blockade of endogenous PI3K within the hippocampus impairs memory

To investigate whether insulin signaling is involved in endogenous hippocampal cognitive processes, we administered either LY294002 or wortmannin, alone, prior to spatial memory testing. Both drugs significantly impaired performance relative to vehicle-injected controls (Fig. 2d); injection of vehicle alone did not affect performance. We interpret the effects of PI3K inhibitors in reversing the effects of insulin, and impairing memory when given alone, as being due to actions on a common pathway. However, these data might possibly have been a case of two independent mechanisms acting to cancel out; hence, we confirmed that (as in other tissues) insulin administration to the hippocampus causes activation of the PI3K pathway, using phosphorylation of the PI3K pathway intermediate Akt (Fig. 3) as a proxy for PI3K activity. As predicted, intrahippocampal administration of 100 μ U insulin produced a local increase of roughly 80% in Akt phosphorylation compared to either control-treated animals or the contralateral hippocampus of insulin-treated animals.

3.4. Selective blockade of intrahippocampal insulin impairs memory performance

The fact that administration of exogenous insulin to the hippocampus enhances memory performance does not categorically show a role for endogenous insulin in memory processes. To further confirm the specificity of memory enhancement by intrahippocampal insulin, and to confirm that insulin is an endogenous modulator of spatial memory processes, we blocked intrahippocampal insulin signaling using hippocampal microinjection of the small (5kD) selective anti-insulin antibody-like protein ab31906 (Abcam). Delivery of ab31906 to the left hippocampus impaired alternation performance (Fig. 2e); no effect was seen with administration of a control antibody-like protein with affinity for ErbB2.

3.5. Insulin and local metabolism

To investigate whether, *in vivo*, insulin affects local intrahippocampal metabolism, we used microdialysis both to sample the extracellular fluid (ECF) of the left hippocampus and as a means for delivery of insulin. Delivery of 10 μ U/min into the left hippocampal ECF produced a rapid elevation of local ECF lactate to ~130% of baseline, accompanied by a drop in ECF glucose to ~75% of baseline (Fig. 4). The alteration in concentration of both glucose and lactate persisted for the 30 min duration of the experiment.

3.6. Systemic insulin resistance impairs insulin's actions within the hippocampus

To determine the impact of systemic insulin resistance on the actions of insulin within the hippocampus, we used the diet-induced obese rat model of T2DM (DIO (Levin, Dunn-Meynell, Balkan, & Keeseey, 1997); see Section 2). Diabetic animals were significantly hyperglycemic and hyperinsulinemic compared to their diet-resistant (DR) controls who did not differ from animals fed regular chow (random sampling of plasma glucose after 4 h fast (mean $\pm$ s.e.m.) 14.7 ± 0.9 mM for DIO vs. 6.0 ± 0.3 for DR, $t(38) = 9.7$, $p < 0.0001$; plasma insulin 1559 ± 153 pM for DIO vs. 436 ± 27 pM for DR, $t(37) = 7.22$, $p < 0.0001$; $n = 20$ for glucose samples and DR insulin, $n = 19$ for DIO insulin). The dose-response curve for baseline spatial memory performance and for enhancement of memory by insulin in DR animals closely resembled that of control animals, despite eating the same diet as the diabetic rats. In contrast, diabetic animals showed a significant impairment in baseline memory performance and a shift to the right in the dose-response curve for insulin enhancement (Fig. 5). No treatment altered motor activity,

measured by number of maze arms entered during testing (data not shown). Further, DIO animals had no significant change in hippocampal ECF glucose or lactate in response to inclusion of 10 μ U/min insulin in microdialysis perfusate (Fig. 6); DR animals' ECF changes were not different to those of controls (not shown).

4. Discussion

Insulin is well-recognized to be the primary regulator of peripheral glucose uptake and metabolism. Within the CNS, insulin has been primarily studied as a regulator of food intake and energy balance via actions within the hypothalamus. However, recent studies have suggested that insulin might also be able to modulate cognitive functions including memory, perhaps via actions within the hippocampus. The current study confirms this hypothesis, and shows that insulin acts directly within the rat hippocampus to enhance memory. Our results show that acute administration of insulin into the hippocampus improves spatial memory in a PI3K-dependent manner. In contrast to the hypothalamus, where blockade of endogenous PI3K activation prevents modulation of food intake by insulin but has no independent effect on food intake (Niswender et al., 2003), administration of PI3K inhibitors alone to the hippocampus impaired memory function even in the absence of exogenous insulin administration, suggesting a physiological role for endogenous, intrahippocampal insulin in hippocampal memory processes. This possibility was confirmed using small, antibody-like peptides to produce a specific blockade of endogenous intrahippocampal insulin, which again impaired memory performance. Importantly, the insulin-induced enhancement of memory performance occurred at a dose within the range of reported measurements of basal (i.e. non food-stimulated) hippocampal extracellular insulin levels (McNay, Green, & Craft, 2006), further supporting a physiological role for insulin in hippocampal memory processes. Determination of the physiological range for interneuronal insulin levels is technically challenging, but the available data reported by McNay, Green et al., (2006) use a microdialysis method specifically developed for interneuronal measurement of peptides and are reassuringly consistent with measurements of insulin in the rat CSF (Stein et al., 1987).

Local insulin delivery also causes rapid and marked changes in hippocampal ECF glucose and lactate concentration, consistent with increased local glucose transport and glycolytic metabolism. These findings are in keeping with previous work showing that performance on hippocampally-mediated spatial memory tasks is limited by glucose availability, and that provision of additional glucose both increases ECF lactate and enhances performance (McNay & Sherwin, 2004a; McNay et al., 2000; McNay et al., 2001; McNay, Williamson, McCrimmon & Sherwin 2006). Our data suggest that *in vivo*, insulin acts directly on the hippocampus to modulate local fuel metabolism. This finding stands in contrast to the traditional model of the brain as an insulin-insensitive organ, but is supported by recent *in vitro* experiments suggesting that hippocampal neurons, in particular, may be metabolically responsive to insulin (Benomar et al., 2006; Duarte, Proenca, Oliviera, Santos, & Rego, 2006) as well as by evidence that insulin administration increases translocation of GluT 4 to the membrane of hippocampal neurons (Reagan, 2005). In the current study, direct delivery of insulin to brain regions outside the hippocampus had no effect on memory performance, consistent with the hypothesized specificity of insulin's actions within the hippocampus. Our data confirm activation of the PI3K signaling cascade as the mechanism by which insulin's actions are transduced within the hippocampus, consistent with results from other tissues. Taken together, our data suggest that insulin may act to support memory processes within the hippocampus by increasing local glucose metabolism. Spatial memory testing such as that used here is known to be limited by glucose metabolism within the hippocampus (McNay et al., 2000), with provision of additional glucose leading to improved performance with an inverted-U dose-response curve similar to that seen here for insulin. One likely mechanism by which insulin acts to support hippocampal memory processes, then, would be via an increase in cell-surface GluT4 leading to increased

intracellular glucose availability; this would also be consistent with the acute increase in lactate observed in the present data. Insulin might also for instance act via alteration of hippocampal synaptic plasticity, as seen in the *in vitro* studies discussed above.

We also examined the impact of high-fat diet-induced T2DM. A high-fat diet alone, in the absence of systemic hyperinsulinemia or increased body mass, did not affect memory performance. However, DIO animals showed impaired memory performance in the control (untreated) condition, and a shift to the right in the dose–response curve for enhancement of performance by intrahippocampal insulin. Further, administration of insulin at a dose which markedly altered ECF glucose and lactate in control and DR animals had no effect on ECF glucose or lactate in DIO animals. Motor performance of DIO animals did not differ from that of other groups. These data suggest that systemic insulin resistance extends to impairment of insulin signaling within the hippocampus, consistent with mRNA measurements in the temporal lobe of high-fat-fed mice (Moroz et al., 2008); a recent study (Clegg et al., 2005) has shown that a similar impairment of central insulin signaling in DIO animals exists within the hypothalamus, and induction of insulin resistance via a high-fructose diet in the hamster showed similar findings (Mielke et al., 2005), suggesting that this may be a more general finding with regard to insulin's actions within the brain. Our data are somewhat in contrast to those of Schubert et al. (2004), who showed little or no effect of genetic deletion, in the mouse, of neuronal insulin receptors on water maze performance or basal brain metabolism; there are, though, many possible explanations for these differences including species, long-term effects of genetic deletion, increased glucose availability following water maze stress, actions of insulin on glia rather than neurons, and so on. Conversely, our data compare intriguingly to recent data from Stranahan and colleagues, showing that a high-fat, high-fructose diet contributes to cognitive and synaptic impairment in middle-aged rats (Stranahan, Norman et al., 2008), and suggesting that this may in part be via effects on circulating glucocorticoids (Stranahan, Arumugam et al., 2008); further studies will be needed to confirm the exact mechanisms by which systemic insulin resistance modulates hippocampal memory processes.

The risk factor for AD in patients with T2DM is several-fold higher than that of age-matched non-diabetic control patients (Leibson et al., 1997), and cognitive performance in aged humans is directly correlated to metabolic control (Hall, Gonder-Frederick, Chewning, Silveira, & Gold, 1989). Insulin sensitivity in humans declines with age even in the absence of T2DM (Fink, Kolterman, Griffin, & Olefsky, 1983), and hence reduced central insulin signaling might play an important role in age-related cognitive decline, as well as in cognitive deficits accompanying e.g. Alzheimer's disease. In keeping with this possibility, acute delivery of intranasal insulin has been reported to partially reverse cognitive deficits in aged human patients (Reger et al., 2006; Stockhorst, de Fries, Steingruber, & Scherbaum, 2004), and the abnormal reduction in hippocampal glucose use seen in the hippocampus of AD patients is accompanied by severe impairment to hippocampal insulin signal transduction (Frolich et al., 1998; Hoyer, 2004; Steen et al., 2005). In addition, it appears that chronic hyperinsulinemia is directly linked to abnormal brain amyloid accumulation (Ho et al., 2004), a defining marker for AD. Thus, the effects of diminished hippocampal insulin signaling may extend beyond impaired glucose metabolism. The present findings suggest that insulin, much like glucose, is a mandatory component of hippocampal memory processes, enhances cognitive performance when acutely elevated (e.g. after a meal, an ecologically salient memory stimulus), but is deleterious if chronically elevated. Our data strongly support the hypothesis that central insulin resistance may be an important contributor to the cognitive deficits associated with T2DM, age-related cognitive decline and dementia.

Acknowledgments

We would like to thank Yuyan Ding for expert surgical assistance, and Dr. Nicole Sanders for valuable commentary on the manuscript; Drs. Barry Levin and Charles Mobbs also offered helpful and insightful commentary. This work was supported by a Junior Faculty Award from the American Diabetes Association to ECM and by the NIH (DK075772 to JB; DK 45735 to RSS; DK077106 to ECM). Portions of this work have been presented previously (ECM, RJM and RSS, Society for Neuroscience Annual Meeting, 2004).

References

- Babri A, Badie H, Khamenei S, Seyedlar M. Intrahippocampal insulin improves memory in a passive-avoidance task in male wistar rats. *Brain and Cognition* 2007;64:86–91. [PubMed: 17349729]
- Banting F, Best C. The internal secretion of the pancreas. *Journal of Laboratory and Clinical Medicine* 1922;7:251–266.
- Benomar Y, Naour N, Auborg A, Bailleux V, Gertler A, Djiane J, et al. Insulin and leptin induce Glut4 plasma membrane translocation and glucose uptake in a human neuronal cell line by a PI 3-kinase dependent mechanism. *Endocrinology* 2006;147:2550–2556. [PubMed: 16497805]
- Clegg D, Benoit S, Reed J, Sc W, Dunn-Meynell AA, Levin BE. Reduced anorexi effects of insulin in obesity-prone rats fed a moderate fat diet. *American Journal of Physiology* 2005;288:R981–R986. [PubMed: 15604298]
- Conrad CD, Lupien SJ, McEwen BS. Support for a bimodal role for type II adrenal steroid receptors in spatial memory. *Neurobiology of Learning & Memory* 1999;72:39–46. [PubMed: 10371714]
- Craft S, Newcomer J, Kanne S, Dagogo-Jack S, Cryer P, Sheline Y, et al. Memory improvement following induced hyperinsulinemia in Alzheimer's disease. *Neurobiology of Aging* 1996;17:123–130. [PubMed: 8786794]
- Craft S, Watson GS. Insulin and neurodegenerative disease: Shared and specific mechanisms. *Lancet Neurology* 2004;3:169–178. [PubMed: 14980532]
- Dember, WN. Spontaneous alternation. Springer; New York: 1989.
- Dore S, Kar S, Rowe W, Quirion R. Distribution and levels of 125I-IGF1, 125I-IGF2 and 125I-insulin receptor binding sites in the hippocampus of aged memory-unimpaired and -impaired rats. *Neuroscience* 1997;80:1033–1040. [PubMed: 9284058]
- Duarte A, Proenca T, Oliveira C, Santos M, Rego A. Insulin restores metabolic function in cultured cortical neurons subjected to oxidative stress. *Diabetes* 2006;55:2863–2870. [PubMed: 17003354]
- Figlewicz D, Bennett J, Evans S, Kaiyala K, Sipols A, Benoit S. Intraventricular insulin and leptin reverse place preference conditioning with high-fat diet in rats. *Behavioral Neuroscience* 2004;118:479–487. [PubMed: 15174925]
- Figlewicz D, Szot P. Insulin stimulates membrane phospholipid metabolism by enhancing endogenous alpha 1-adrenergic activity in the rat hippocampus. *Brain Research* 1991;550:101–107. [PubMed: 1653632]
- Fink RI, Kolterman OG, Griffin J, Olefsky JM. Mechanisms of insulin resistance in aging. *Journal of Clinical Investigation* 1983;71:1523–1535. [PubMed: 6345584]
- Frolich L, Blum-Degen D, Bernstein H-G, Engelsberger S, Humrich J, Laufer S, et al. Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *Journal of Neural Transmission* 1998;105:423–438. [PubMed: 9720972]
- Gispén W, Biessels G. Cognition and synaptic plasticity in diabetes mellitus. *Trends in Neuroscience* 2000;542–549.
- Gold P. Glucose and age-related changes in memory. *Neurobiology of Aging* 2005;26S:S60–64.
- Hall J, Gonder-Frederick L, Chewing W, Silveira J, Gold P. Glucose enhancement of performance on memory tests in young and aged humans. *Neuropsychologia* 1989;27:1129–1138. [PubMed: 2812297]
- Hasselbalch S, Knudsen G, Videbaek C, Pinborg L, Schmidt J, Holm S, et al. No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism. *Diabetes* 1999;48:1915–1921. [PubMed: 10512354]

- Ho L, Qin W, Pompl P, Xiang Z, Wang J, Zhao Z, et al. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. *FASEB Journal* 2004;18:902–904. [PubMed: 15033922]
- Hoyer S. Glucose metabolism and insulin receptor signal transduction in Alzheimer disease. *European Journal of Pharmacology* 2004;115–125. [PubMed: 15094078]
- Izumi Y, Yamada K, Matsukawa M, Zorumski C. Effects of insulin on LTP in hippocampal slices from diabetic rats. *Diabetologia* 2003;46:1007–1012. [PubMed: 12827244]
- Kern W, Peters A, Freuhwald-Schultes B, Deininger E, Born J, Fehm H. Improving influence of insulin on cognitive functions in humans. *Clinical Neuroendocrinology* 2001;74:270–280.
- Leibson C, Rocca W, Hanson V, Cha R, Kokmen E, O'Brien P, et al. Risk of dementia among persons with diabetes mellitus: A population-based cohort study. *American Journal of Epidemiology* 1997;145:301–308. [PubMed: 9054233]
- Levin BE, Dunn-Meynell AA, Balkan B, Keesey R. Selective breeding for diet-induced obesity and insulin resistance in Sprague-Dawley rats. *American Journal of Physiology* 1997;273:R725–R730. [PubMed: 9277561]
- Long J, Davis B, Garofalo P, Spangler E, Ingram D. Complex maze performance in young and aged rats: Response to glucose treatment and relationship to blood insulin and glucose. *Physiology & Behavior* 1992;51:411–418. [PubMed: 1313593]
- McEwen BS, Reagan L. Glucose transporter expression in the central nervous system: Relationship to synaptic function. *European Journal of Pharmacology* 2004;90:13–24. [PubMed: 15094070]
- McNay EC, Fries TM, Gold PE. Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97:2881–2885. [PubMed: 10706633]
- McNay EC, Gold PE. Memory modulation across neural systems: Intraamygdala glucose reverses deficits caused by intraseptal morphine on a spatial task but not on an aversive task. *Journal of Neuroscience* 1998;18:3853–3858. [PubMed: 9570814]
- McNay EC, Gold PE. Food for thought: Fluctuations in brain extracellular glucose provide insight into the mechanisms of memory modulation. *Cognitive and Behavioural Neuroscience Reviews* 2002;1:264–280.
- McNay, EC.; Green, P.; Craft, S. Program # 16.4: Insulin and brain beta-amyloid: in vivo microdialysis studies; Society for Neuroscience Annual Meeting; Atlanta. 2006.
- McNay EC, McCarty RC, Gold PE. Fluctuations in brain glucose concentration during behavioral testing: Dissociations between brain areas and between brain and blood. *Neurobiology of Learning & Memory* 2001;75:325–337. [PubMed: 11300738]
- McNay EC, Sherwin RS. Effect of recurrent hypoglycemia on spatial cognition and cognitive metabolism in normal and diabetic rats. *Diabetes* 2004a;53:418–425. [PubMed: 14747293]
- McNay EC, Sherwin RS. From artificial cerebro-spinal fluid (aCSF) to artificial extracellular fluid (aECF): Microdialysis perfusate composition effects on in vivo brain ECF glucose measurements. *Journal of Neuroscience Methods* 2004b;132:35–43. [PubMed: 14687673]
- McNay EC, Williamson A, McCrimmon RJ, Sherwin RS. Cognitive and neural hippocampal effects of long-term moderate recurrent hypoglycemia. *Diabetes* 2006;55:1088–1095. [PubMed: 16567533]
- Mielke J, Taghibiglou C, Liu L, Zhang Y, Jia Z, Adeli K, et al. A biochemical and functional characterization of diet-induced brain insulin resistance. *Journal of Neurochemistry* 2005;93:1568–1578. [PubMed: 15935073]
- Moosavi M, Naghdi N, Choopani S. Intra CA1 insulin microinjection improves memory consolidation and retrieval. *Peptides* 2007;28:1029–1034. [PubMed: 17360072]
- Moosavi M, Naghdi N, Maghsoudi N, Asl S. The effect of intrahippocampal insulin microinjection on spatial learning and memory. *Hormones and Behavior* 2006;50:748–752. [PubMed: 16890939]
- Moroz N, Tong M, Longato L, Xu H, Monte S. M. dl. Limited Alzheimer-type neurodegeneration in experimental obesity and type 2 diabetes mellitus. *Journal of Alzheimer's Disease* 2008;15:29–44.
- Niswender K, Morrison C, Clegg D, Olson R, Baskin D, Myers MJ, et al. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: A key mediator of insulin-induced anorexia. *Diabetes* 2003;52:227–231. [PubMed: 12540590]

- Park CR, Seeley RJ, Craft S, Woods SC. Intracerebroventricular insulin enhances memory in a passive avoidance task. *Physiology and Behaviour* 2000;68:509–514.
- Plum L, Belgardt B, Bruning J. Central insulin action in energy and homeostasis. *JCI* 2006;116:1761–1766. [PubMed: 16823473]
- Pocai A, Lam T, Gutierrez-Juarez R, Obici S, Schwartz G, Bryan J, et al. Hypothalamic K(ATP) channels control hepatic glucose production. *Nature* 2005;434:1026–1031. [PubMed: 15846348]
- Reagan L. Neuronal insulin signal transduction mechanisms in diabetes phenotypes. *Neurobiology of Aging* 2005;26S:S56–59.
- Rechler MM, Nissley SP. The nature and regulation of the receptors for insulin-like growth factors. *Annual Review of Physiology* 1985;47:425–442.
- Reger M, Watson G, Frey W, Baker L, Cholerton B, Keeling M, et al. Effects of intranasal insulin on cognition in memory-impaired older adults: Modulation by APOE genotype. *Neurobiology of Aging* 2006;27:451–458. [PubMed: 15964100]
- Sarter M, Bodewitz G, Steckler T. 2-[3H]deoxyglucose uptake patterns in rats exploring a six-arm radial tunnel maze: Differences between experienced and nonexperienced rats. *Behavioral Neuroscience* 1989;103:1217–1225. [PubMed: 2610914]
- Schubert M, Gautam D, Surjo D, Ueki K, Baudler S, Schubert D, et al. Role for neuronal insulin resistance in neurodegenerative diseases. *PNAS* 2004;101:3100–3105. [PubMed: 14981233]
- Schulinkamp R, Pagano T, Hung D, Raffa R. Insulin receptors and insulin action in the brain: Review and clinical implications. *Neuroscience and Biobehavioral Reviews* 2000;24:855–872. [PubMed: 11118610]
- Schwartz M, Figlewicz D, Baskin D, Woods S, Porte DJ. Insulin in the brain: A hormonal regulator of energy balance. *Endocrine Reviews* 2000;13:387–414. [PubMed: 1425482]
- Sequist E, Damberg G, Tkac I, Gruetter R. The effect of insulin on in vivo cerebral glucose concentrations and rates of glucose transport/metabolism in humans. *Diabetes* 2001:50.
- Spanwick D, Smith MA, Mirshamshi S, Routh VH, Ashford ML. Insulin activates ATP-sensitive K⁺ channels in hypothalamic neurons of lean, but not obese, rats. *Nature Neuroscience* 2000;3:757–758.
- Steen E, Terry B, Rivera E, Cannon J, Neely T, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease: Is this type 3 diabetes? *Journal of Alzheimer's Disease* 2005;7:63–80.
- Stefani MR, Gold PE. Intrahippocampal infusions of k-atp channel modulators influence spontaneous alternation performance: Relationships to acetylcholine release in the hippocampus. *Journal of Neuroscience* 2001;21:609–614. [PubMed: 11160440]
- Stein LJ, Dorsa DM, Baskin DG, Figlewicz DP, Porte DJ, Woods SC. Reduced effect of experimental peripheral hyperinsulinemia to elevate cerebrospinal fluid insulin concentrations of obese Zucker rats. *Endocrinology* 1987;121:1611–1615. [PubMed: 3311715]
- Stockhorst U, de Fries D, Steingruber H-J, Scherbaum W. Insulin and the CNS: Effects on food intake, memory, and endocrine parameters and the role of intranasal insulin administration in humans. *Physiology & Behavior* 2004;83:47–54. [PubMed: 15501490]
- Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nature Neuroscience* 2008a;11:309–317.
- Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, et al. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus* 2008b;18:1085–1088. [PubMed: 18651634]
- van der Heide LP, Kamal A, Artola A, Gispen WH, Ramakers GM. Insulin modulates hippocampal activity-dependent synaptic plasticity in a N-methyl-D-aspartate receptor and phosphatidylinositol-3-kinase-dependent manner. *Journal of Neurochemistry* 2005;94:1158–1166. [PubMed: 16092951]
- Vannucci S, Koehler-Stec E, Kang L, Reynolds T, Clark R, Simpson I. GluT4 glucose transporter expression in rodent brain: Effect of diabetes. *Brain Research* 1998;797:1–11. [PubMed: 9630471]
- Watson GS, Craft S. Modulation of memory by insulin and glucose: Neuropsychological observations in Alzheimer's disease. *European Journal of Pharmacology* 2004;490:97–113. [PubMed: 15094077]

- Winocur G, Gagnon S. Glucose treatment attenuates spatial learning and memory deficits of aged rats on tests of hippocampal function. *Neurobiology of Aging* 1998;19:233–241. [PubMed: 9661998]
- Winocur G, Greenwood C, Pirolli G, Grillo C, Reznikov L, Reagan L, et al. Memory impairment in obese Zucker rats: An investigation of cognitive function in an animal model of insulin resistance and obesity. *Behavioral Neuroscience* 2005;119:1389–1395. [PubMed: 16300445]
- Woods S, Schwartz M, Baskin D, Seeley R. Food intake and the regulation of body weight. *Annual Review of Psychology* 2000;51:255–277.
- Yu C, Cresswell J, Loffler M, Bogan J. The glucose transporter 4-regulating protein TUG is essential for highly insulin-responsive glucose uptake in 3T 3-L1 adipocytes. *Journal of Biological Chemistry* 2007;282:7710–7722. [PubMed: 17202135]
- Zhao W-Q, Chen H, Quon M, Alkon D. Insulin and the insulin receptor in experimental models of learning and memory. *European Journal of Pharmacology* 2004;490:71–81. [PubMed: 15094074]
- Zhao W-Q, Chen H, Xu H, Moore E, Meiri N, Quon M, et al. Brain insulin receptors and spatial memory: Correlated changes in gene expression, tyrosine phosphorylation, and signalling molecules in the hippocampus of water maze trained rats. *Journal of Biological Chemistry* 1999;274:34893–34902. [PubMed: 10574963]

Fig. 1.

Memory performance (percent 4/5 alternation) in control animals (on standard Purina chow) following administration of intrahippocampal insulin. Asterisks indicate significant group performance enhancement of animals in the 100 μ U and 1 mU insulin groups, compared to animals in control group, as determined by Dunnett's *post hoc* tests, both $p < 0.01$. ANOVA: $F = 16.44$, $p < 0.0001$. Error bars indicate s.e.m. Figures within each bar indicate N of group.

Fig. 2.

(a) Memory performance following administration of (i) aECF, (ii) 100 μ U intrahippocampal insulin, (iii) 100 μ U intrastratial insulin, or (iv and v) intrahippocampal IGF-1. Asterisk indicates significant group performance enhancement of animals in the 100 μ U intrahippocampal insulin group, only, compared to control animals; ANOVA $F = 6.99$, $p < 0.0002$. (b) Memory performance following administration of 100 μ U intrahippocampal insulin either alone or co-administered with the PI3K inhibitors wortmannin (39.7 μ M) or LY294002 (20.2 mM). Asterisk indicates significant group performance enhancement of animals in the 100 μ U intrahippocampal insulin group only; ANOVA $F = 15.46$, $p < 0.0001$. (c) Memory performance following administration of intrahippocampal diazoxide. Asterisk indicates significant impairment of animals in the 2.5 mM diazoxide group; ANOVA $F = 9.02$, $p < 0.0001$. (d) Memory performance following administration of intrahippocampal wortmannin or LY294002, doses as in b. Asterisks indicate significant group impairment of animals in both

drug groups compared to control animals; ANOVA $F = 9.59$, $p < 0.0001$. (e) Memory performance following administration of intrahippocampal small antibody-like proteins (Affibodies, Abcam; 0.5 μg in 0.5 μL) against either insulin or the non-brain protein ErbB2. Asterisk indicates significant group impairment of animals in the anti-insulin group compared to those in both other groups; ANOVA $F = 21.36$, $p < 0.0001$. Error bars indicate s.e.m. Figures within each bar indicate N of group; aECF and 100 μM hippocampal insulin groups are repeated for ease of comparison.

Fig. 3.

Immunoblotting for phosphorylated Akt (P-Akt) in the hippocampus following unilateral administration of either 100 μ U insulin or aECF (control animals). Quantitative densitometry was normalized to set the mean for the ipsilateral-control at 1.0, with group means and SEM being 1.03 ± 0.080 (contralateral insulin), 0.87 ± 0.229 (contralateral control), 1.77 ± 0.104 (ipsilateral insulin), and 1.00 ± 0.098 (ipsilateral control); $N = 6$ for each group. Administration of insulin caused significant elevation of P-Akt in the ipsilateral samples compared to all other groups; ANOVA $F = 8.29$, $p < 0.001$. A similar analysis of total Akt immunoblots showed insulin did not cause any significant change in total Akt in ipsilateral samples compared to all other groups.

Fig. 4. Extracellular hippocampal glucose (solid line) and lactate (dashed line) in control animals following inclusion of insulin ($10 \mu\text{U}/\text{min}$) in the microdialysis perfusate. Error bars indicate s.e.m. $N = 8$.

Fig. 5.

Memory performance in control (white bars; reproduced from Fig. 1 for convenience), DR (green bars) and DIO (red bars) animals following administration of intrahippocampal insulin. Asterisks indicate significant group performance impairment of animals in the DIO group compared to DR animals, at baseline [$t(14) = 3.21, p < 0.01$] and following administration of 100 µU insulin [$t(14) = 6.10, p < 0.0001$]. Error bars indicate s.e.m. N for control groups as Fig. 1; $N = 8$ for all DIO and DR groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. Extracellular hippocampal glucose (solid line) and lactate (dashed line) in DIO animals following inclusion of insulin ($10 \mu\text{U}/\text{min}$) in the microdialysis perfusate. Error bars indicate s.e.m. $N = 8$.