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Brief communication

Insulin effects on CSF norepinephrine and cognition in Alzheimer's disease

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Abstract

We assessed the effects of induced hyperinsulinemia on plasma and cerebrospinal fluid (CSF) levels of norepinephrine (NE) and on cognition for patients with Alzheimer's disease (AD) and normal older adults. For normal adults, insulin increased plasma and CSF NE levels; also, recall for paraphrased details of a story improved as CSF NE levels increased. Mental control was positively correlated with CSF levels of NE for patients. These findings demonstrate that raising peripheral insulin levels can modulate CNS NE levels and suggest that insulin-stimulated increases in NE may modulate cognitive functions.

Keywords: Aging; Alzheimer's disease; Attention; Amnestic mild cognitive impairment; Cerebrospinal fluid; Insulin; Lumbar puncture; Norepinephrine; Memory

1. Introduction

Recent studies demonstrate that insulin resistance (impaired insulin efficiency) may contribute to the formation of senile plaques and neurofibrillary tangles, the pathophysiological hallmarks of Alzheimer's disease (AD) [7,12]. Impaired insulin activity may also influence other pathophysiological features of AD, such as altered noradrenergic functioning associated with the loss of neurons in the locus coeruleus [16]. For example, insulin reduces norepinephrine (NE) uptake in neuronal cultures and NE transporter mRNA in vivo in rats [1,6]. Thus, changes in insulin levels may modulate synaptic NE levels and behaviors normally associated with noradrenergic activity. Consistent with this notion, intraventricular insulin administration suppresses the acoustic startle reflex in a manner similar to the actions of the NE reuptake inhibitor desipramine [13]. Furthermore, plasma insulin readily crosses the blood brain barrier and raises the level of insulin in cerebrospinal fluid (CSF) [15]. Once in the brain, insulin potentially can alter NE levels, and thus influence attention, a cognitive function supported by noradrenergic activity in the prefrontal cortex [8].

The present study examined the effect of induced hyperinsulinemia with euglycemia on CSF NE levels and on the relationship between cognitive performance and insulin-induced changes in CSF NE. We hypothesized that intravenous insulin administration would raise CSF NE levels for older adults

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with and without AD, and that insulin-induced increases in CSF NE levels would facilitate attention.

2. Subjects and methods

2.1. Subjects

The University of Washington Human Subjects Division approved the study, and written consent was obtained from all participants. A memory-impaired group consisted of four patients with mild AD (NINCDS/ADRDA criteria) and three patients with amnestic mild cognitive impairment (MCI), who have a substantially increased risk for developing AD [11]. Patients who achieved Mini-Mental State Examination (MMSE) scores below 15 during screening were excluded from study participation, and only one patient scored below 20 during the study. A control group consisted of 16 normal older adults. All participants were in good general health. Groups did not differ with respect to age (mean ± S.D. (years): patients (68.7 ± 4.8); controls (68.8 ± 8.4)), gender, education, body mass index, or fasting plasma levels of glucose, insulin, epinephrine, or NE. Baseline MMSE scores (mean ± S.D.) were significantly lower for patients (23.6 ± 6.0) than for controls (29.4 ± 0.7), $F(1, 22) = 9.14$, $P < 0.001$. The 30-min dextrose (20%) disposal rate in response to a fixed insulin dose was calculated to provide an index of insulin sensitivity. The dextrose disposal rate (mean ± S.E.M. (mL)) was lower but not significantly different for patients (33.4 ± 6.9) than normal adults (52.3 ± 6.8), although this is likely due to the small sample size.

2.2. Procedure, assays, and analyses

On separate mornings, fasting subjects received randomized infusions of (1) saline or (2) insulin ($1.0\,\text{mU}\,\text{kg}^{-1}\,\text{min}^{-1}$) accompanied by variable dextrose to maintain plasma glucose at ∼100 mg/dL, as previously described [15]. Ninety minutes after infusions were initiated, subjects completed a 15-min cognitive battery consisting of the MMSE, story recall, and the Stroop color–word interference test. Blood was acquired twice, prior to infusion and prior to cognitive testing. CSF was acquired after testing. In brief, subjects were placed in the lateral decubitus position, and local anesthesia was achieved with lidocaine. A 24-g atraumatic spinal needle was inserted in the L4-5 interspace and 27 mL of CSF was withdrawn, frozen immediately, and stored as 0.5-mL aliquots until assay. Insulin, NE, and epinephrine levels were measured with radioenzymatic or immuno assays.

For story recall, subjects heard a brief narrative and recall was elicited immediately. Verbatim responses (1 point) and paraphrases (1/2 point) were summed to compute verbatim and paraphrase scores, respectively. Accurate responses on MMSE reverse subtraction were summed to compute a mental control score (maximum 5). Story recall and metabolic values were log-transformed. Mental control scores were not transformed due to restricted range. Dependent measures were submitted to a mixed model repeated measures analysis of variance with infusion condition (saline, insulin) as a within-subjects factor and diagnosis (patient, normal) as a between-subjects factor. Using regression analyses, residual scores were calculated for CSF NE levels and for recall scores; values obtained during insulin and saline infusions were the dependent and independent variables, respectively. Larger residual scores reflect greater change in NE or recall due to insulin. Linear relationships were assessed with Pearson correlations. Insulin effects on memory and CSF $A\beta_{42}$ were reported previously for normal adults [15].

3. Results

As expected, insulin administration significantly raised plasma insulin levels for both patients (mean ± S.E.M. (μU/mL) = 8.91 ± 1.08, saline condition; 65.71 ± 3.23, insulin condition) and normal adults (mean ± S.E.M. (μU/mL) = 9.72 ± 0.79, saline condition; 72.35 ± 4.60, insulin condition). In contrast, insulin administration significantly raised CSF insulin levels for normal adults (mean ± S.E.M. (μU/mL) = 1.47 ± 0.22, saline condition; 2.29 ± 0.27, insulin condition), as previously reported [15], but not for patients (mean ± S.E.M. (μU/mL) = 1.89 ± 0.26, saline condition; 2.29 ± 0.78, insulin condition). In the saline condition, plasma and CSF NE levels did not differ by diagnostic group. Insulin significantly raised plasma NE levels ($F(1, 12) = 6.06$, $P = 0.030$; Fig. 1A) and CSF NE levels ($F(1, 14) = 8.61$, $P = 0.011$; Fig. 1B) for normal adults but not for patients. CSF and plasma NE levels were not correlated in either condition for normal adults. In contrast, higher CSF NE levels were associated with higher plasma NE in saline ($r = 0.85$, $P = 0.016$) and insulin ($r = 0.90$, $P = 0.016$) conditions for patients. Insulin administration did not alter plasma or CSF epinephrine levels for either group.

Insulin facilitated story recall for normal adults, as previously reported [15]. For the combined sample, residual paraphrase recall scores increased as residual CSF NE levels increased ($r = 0.48$, $P = 0.028$; Fig. 2A), suggesting that insulin-induced changes in CSF NE facilitated paraphrase recall. This relationship was significant for normal adults alone ($r = 0.53$, $P = 0.044$) but not patients. The association between verbatim and CSF NE residuals was not significant. We also compared correlations between CSF NE residuals and paraphrase residuals versus CSF NE residuals and verbatim residuals ($r = -0.34$, $P = 0.210$) for normal adults [9], and the two correlations differed significantly ($Z = 2.11$, $P = 0.035$). During insulin administration, MMSE mental control scores increased as CSF NE levels increased for the combined patient and control groups ($r = 0.51$, $P = 0.013$), suggesting that higher CSF NE levels may facilitate mental control. This relationship approached significance for patients alone ($r = 0.72$, $P = 0.066$) but not normal adults alone, likely due to a ceiling effect. No effects were observed on the Stroop task.

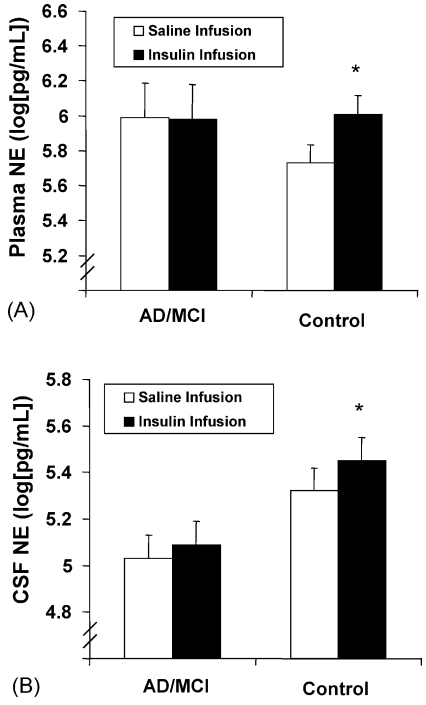

Fig. 1. Effects of insulin infusion on plasma and cerebrospinal fluid (CSF) norepinephrine (NE) levels. Data are depicted as log-transformed means ± S.E.M. (A) Plasma NE levels did not differ by diagnostic group during the saline infusion. Insulin administration raised plasma NE levels for the normal adults ($F(1, 12) = 6.06$, $P = 0.030$) but not patients. Untransformed means ± S.E.M. (pg/mL) for patients are 456.67 ± 107.23 (saline) and 433.33 ± 96.60 (insulin); and for normal adults are 323.85 ± 30.52 (saline) and 434.62 ± 46.75 (insulin). (B) CSF NE levels did not differ by diagnostic group during the saline infusion. Insulin administration raised CSF NE levels for all subjects ($F(1, 20) = 4.41$, $P = 0.049$). By diagnostic group, the insulin-induced increase in CSF NE was significant ($F(1, 14) = 8.61$, $P < 0.011$) for normal adults, but not for patients. Untransformed means ± S.E.M. (pg/mL) for patients are 157.14 ± 13.75 (saline) and 168.57 ± 19.93 (insulin); and for normal adults are 217.33 ± 22.67 (saline) and 249.33 ± 27.02 (insulin). As (B) suggests, insulin induced small but highly reliable changes in CSF NE for normal adults: mean change in CSF NE ± S.E.M. (pg/mL) = 0.10 ± 0.04 (log transformed) and 25.54 ± 8.72 (untransformed).

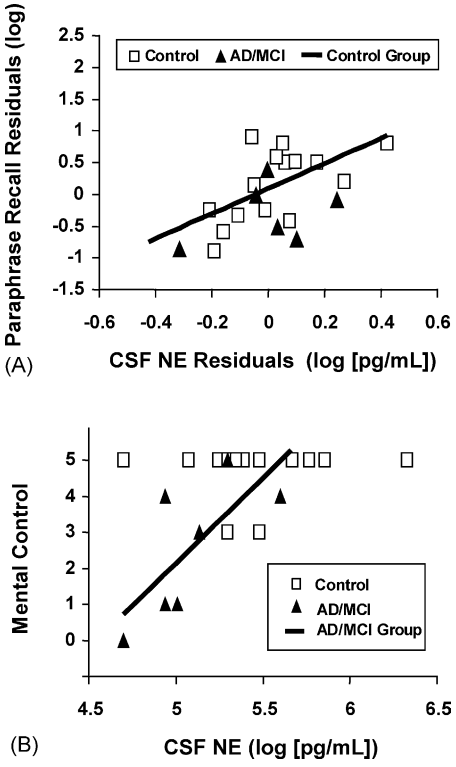

Fig. 2. Relationship of CSF NE levels and cognitive performance. (A) Insulin-induced changes in CSF NE levels were related to changes in paraphrase recall scores. Specifically, higher residual paraphrase scores on story recall were correlated with higher residual CSF NE levels for patients and normal adults together ($r = 0.48$, $P = 0.028$) and for normal adults alone ($r = 0.53$, $P = 0.044$) but not patients alone. (B) During insulin infusion, MMSE mental control scores (maximum 5) improved as CSF NE levels increased for the combined patient and control groups ($r = 0.51$, $P = 0.013$) and for the patient group alone ($r = 0.72$, $P = 0.066$). The control group alone did not show this relationship, likely due to ceiling effects on mental control.

4. Discussion

In the present study, insulin administration significantly increased CSF insulin and NE levels for healthy older adults, consistent with earlier reports that peripheral insulin readily crosses the blood brain barrier where it may reduce neuronal reuptake of NE [1,6]. In contrast, insulin induced smaller non-significant increases in CSF insulin and NE levels for patients. Similarly, we previously reported that plasma and CSF insulin levels were abnormal in a sample of 25 patients with AD, and hypothesized that the relationship between peripheral and central insulin may be disrupted in AD [5]. Our current findings support the notion that insulin transport across the blood brain barrier may be reduced in AD, and further suggest that reduced insulin transport may be associated with reduced brain noradrenergic responses to induced hyperinsulinemia. A second, and perhaps complementary, explanation for our current findings is that brain noradrenergic functions may be impaired in AD. For example, AD is associated with a significant loss of noradrenergic neurons in the locus coeruleus [16]. Therefore, it is possible that NE release in response to sustained hyperinsulinemia is reduced in AD.

We have previously suggested that insulin administration may influence memory via effects on β-amyloid, long-term potentiation, cyclic AMP-response element binding protein, brain-derived neurotrophic factor, and acetylcholine, as well as by effects on glucose disposal [14,15]. In the present study, CSF NE levels were positively related to performance on an immediate story recall task. Paraphrase recall improved as insulin induced higher CSF NE levels for normal adults (Fig. 2), the only group in which insulin increased CSF NE levels. These observations suggest that insulin crossed the blood brain barrier and stimulated an increase in brain NE levels, which, in turn, facilitated immediate paraphrase recall. The distinction between paraphrase and verbatim recall may seem counterintuitive, but memory for gist and verbatim details can be dissociated with a benzodiazepine (anxiolytic) agent. It has been shown that gist but not verbatim memory improves as arousal increases, possibly due to effects on

the amygdala [2]. In the present study, elevated CSF NE may have increased arousal, and thus facilitated paraphrase recall. In contrast, findings were mixed for two attention tasks that activate the frontal lobes, mental control [3] and the Stroop task [10]. In the patient group, higher CSF NE levels during insulin administration were associated with improved mental control, likely due to enhanced noradrenergic activity in the prefrontal cortex [8]. Unexpectedly, we did not find an association between CSF NE levels and performance on the Stroop task for the patient group, possibly due to the small sample or, alternatively, to differences in task demands. For normal adults, CSF levels of NE were not associated with changes in performance on the Stroop task or mental control, likely due to a ceiling effect on these measures.

Taken together, results of this study are consistent with a role for insulin in brain noradrenergic activity; however, two limitations should be raised. First, our patient sample was small, and these results should be confirmed in a larger group of patients. Second, the measures employed in this study were designed to assess the cognitive effects of insulin, which facilitates declarative memory [4,15]. Therefore, future studies would benefit from inclusion of more sophisticated measures of attention. Nevertheless, our findings do show that intravenous insulin administration raises brain NE levels, and these noradrenergic changes are associated with cognitive facilitation.

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