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Brain Insulin Infusion Does Not Augment the Counterregulatory Response to Hypoglycemia or Glucoprivation

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Abstract

Although high dosages of insulin can cause hypoglycemia, several studies suggest that increased insulin action in the head may paradoxically protect against severe hypoglycemia by augmenting the sympathoadrenal response to hypoglycemia. We hypothesized that a direct infusion of insulin into the third ventricle and/or the mediobasal hypothalamus (MBH) would amplify the sympathoadrenal response to hypoglycemia. Nine week-old male rats had insulin (15 mU) or artificial cerebrospinal fluid (aCSF- control) infused bilaterally into the MBH or directly into the third ventricle. During the final two hours of the brain insulin or aCSF infusions, the counterregulatory response to either a hyperinsulinemic hypoglycemic (~50 mg/dl) clamp or to a 600 mg/kg IV bolus of 2-deoxy-glucose (2DG) was measured. 2DG was used to induce a glucoprivic response without peripheral insulin infusion. In response to insulin-induced hypoglycemia, epinephrine rose more than 60-fold, norepinephrine rose more than 4-fold, glucagon 8 fold, and corticosterone almost 2-fold, but these increments were not different in aCSF versus insulin treatment groups with either ICV or bilateral MBH insulin protocols. ICV insulin infusion stimulated insulin signaling as noted by a 5-fold increase in AKT phosphorylation. In the absence of systemic insulin infusion, 2DG-induced glucopenia resulted in an equal counterregulatory response with brain aCSF and insulin infusions. Under the conditions studied, although insulin infusion acted to stimulate hypothalamic insulin signaling neither intrahypothalamic nor intracerebroventricular insulin infusion augmented the counterregulatory response to hypoglycemia nor to 2DG-induced glucoprivation. Therefore, it is proposed that the previously noted acute actions of insulin to augment the sympathoadrenal response to hypoglycemia are likely mediated via mechanisms exterior to the central nervous system.

Introduction

The greatest clinical challenge associated with the use of insulin in the management of diabetes is to fully correct hyperglycemia without causing hypoglycemia. Counterregulation in response to hypoglycemia is impaired in insulin-treated diabetic patient who lack a glucagon response¹. Diabetic patients are usually solely dependent on their sympathoadrenal response to prevent severe hypoglycemia. Unfortunately, even a single episode of hypoglycemia has been shown

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to reduce the sympathoadrenal response to future episodes of hypoglycemia ^{2;3}. Diabetic patients who develop this impaired sympathoadrenal response are at markedly increased risk for severe, life-threatening hypoglycemia ^{1;4}. Thus, extensive research efforts have focused on elucidating the mechanisms which underlie this impaired sympathoadrenal response with the hope of finding novel approaches to preventing and/or restoring the impaired counterregulatory response to hypoglycemia.

There has been much debate about whether insulin per se may increase the sympathoadrenal response to hypoglycemia ⁵. At equivalent levels of hypoglycemia, some studies have demonstrated that increased insulin levels augment the sympathoadrenal response in non-diabetic ^{6;7} and diabetic humans ^{8;9} whereas other studies failed to demonstrate an effect of increased insulin levels to alter the sympathoadrenal response to hypoglycemia in non-diabetic ^{10;11} and diabetic humans ^{10;12;13}. Paradoxically, one study has demonstrated the increased insulin levels significantly diminished the sympathoadrenal response ¹⁴. The site of insulin action could not be determined from the above reports. Hypoglycemia experiments in dogs demonstrated that a selective increase in carotid and vertebral artery insulin levels profoundly augmented the epinephrine response suggesting that insulin acts in the head to augment the sympathoadrenal response to hypoglycemia ^{15;16}. Supporting the notion that insulin may be acting in the central nervous system to augment the sympathoadrenal response to hypoglycemia, brain/neuron-specific insulin receptor knockout (NIRKO) mice have a blunted sympathoadrenal response to insulin-induced hypoglycemia ¹⁷. Consistent with reports that the mediobasal hypothalamus (comprising of the ventromedial hypothalamus and the arcuate nucleus) are primary sites of glucose sensing ¹⁸⁻²⁰ and insulin action ^{21;22}, NIRKO mice have been shown to have changes in glucose transporter expression in the mediobasal hypothalamus ²³. Taken together, these studies suggest that the cerebral circulation or the central nervous system, and perhaps the mediobasal hypothalamus, may be the sites where increased insulin levels act to augment counterregulation. Therefore, it was hypothesized that a direct infusion of insulin into brain (ie, into the cerebrospinal fluid within the third ventricle or directly into the parenchyma of the mediobasal hypothalamus) would amplify the sympathoadrenal response to hypoglycemia.

Research Design and Methods

Animals and procedures

Nine-week-old male Sprague-Dawley rats were fed a standard rat chow, and housed on a 12-h/12-h day/night cycle. Two weeks prior to each study, the animals were anesthetized with isoflurane, and microinjection cannula (Plastics One Inc., Roanoke, VA) were inserted into the mediobasal hypothalamus or the third ventricle. At the border of the ventromedial hypothalamus and the arcuate nucleus, the mediobasal hypothalamus was targeted by bilateral cannulae (coordinates from bregma: posterior 2.8 mm, targeted depth 10.1 mm, lateral +/- 0.6 mm). For intracerebroventricular (ICV) injections, a solitary ICV cannula was inserted 2.8 mm posterior to bregma, on the suture line, to a targeted depth 10.1 mm. After a one week recovery period, the animals were anesthetized with ketamine/xylazine (87/13.4 mg/kg IP) and microcatheters (Braintree Scientific, Braintree, MA) were implanted into the left carotid artery and right jugular vein. The animals were then allowed approximately one week to recover prior to the experiment and a recovery of pre-surgery body weight was used as an index of the animals' health. Animals were excluded from analysis if the intrahypothalamic or ICV cannulae were not positioned properly as determined on posthumous examination with Evans blue dye (0.0075%). All animal procedures were approved by the Animal Studies Committee of Washington University.

Hyperinsulinemic hypoglycemic clamp protocol

Following a 15 hours fast, each animal was briefly anesthetized with isoflurane (<5 minutes) in order to insert and secure the internal brain cannulae to the designated depth as well as to attach the vascular catheters. Insulin (total dose of 15 mU in aCSF) or vehicle (aCSF) was infused into the brain for five hours via the bilaterally implanted intrahypothalamic cannulae at 0.025 mU/min (0.05 μ l/min bilaterally) whereas insulin was infused into the ICV cannula at 0.05 mU/min (0.05 μ l/min). Data from the literature indicated that such a pharmacologic dose of insulin would be optimal to observe an augmentation of the counterregulatory response to hypoglycemia because pharmacologic insulin doses are necessary to elicit insulin signaling events in the hypothalamus²⁴ and an insulin-induced augmentation of the counterregulatory response is best observed when insulin levels are raised 100-600 fold^{6;25-27}. Following a 3 hour basal rest period in which the animals were not handled, baseline arterial blood samples were obtained in awake, unrestrained rats. During the final two hours of the brain insulin or aCSF infusions, hypoglycemia was induced with an intravenous infusion of regular human insulin (Lilly, Indianapolis, IN) diluted in phosphate buffered saline with 1% BSA. Systemic insulin was administered at a low dose in the intrahypothalamic experiments (50 mU/kg bolus and 5 mU.kg⁻¹.min⁻¹) and at a high dose in the ICV experiments (150 mU/kg bolus and 15 mU.kg⁻¹.min⁻¹). Arterial blood samples (0.3 μ l) were drawn at 10 minute intervals to measure blood glucose. A 50% dextrose infusion was adjusted to achieve blood glucose levels of ~50 mg/dl within 30 minutes. When the blood glucose reached ~50 mg/dl, the clock was reset to 0, and blood samples were collected at 0, 30, 60, 90, and 120 minutes. Red blood cells were re-suspended in heparinized (20 U/ml) saline and re-infused following each blood sample to avoid anemia and volume depletion. The dextrose infusion was titrated to maintain blood glucose at 40-50 mg/dl throughout the clamp. At the end of the experiment, some rats were briefly anesthetized with isoflurane in order to obtain samples of cerebrospinal fluid (CSF) by cervical spine needle insertion.

2DG-induced glucoprivation protocol

A similar protocol as described above for the hyperinsulinemic hypoglycemic clamp protocol was used except that in lieu of systemic insulin and glucose administration, a bolus of 2-deoxyglucose (600 mg/kg IV) was given 3 hours after insulin or aCSF was infused into the two intrahypothalamic cannulae. A basal blood sample was taken immediately before the 2DG bolus and then at 30, 60, 90, and 120 minutes following the 2DG bolus.

Insulin Signaling

To determine whether the dose of insulin was effective in eliciting insulin signaling events in the hypothalamus, western blot analysis was performed to quantify AKT phosphorylation given its relatively robust response to insulin when using low specific protein content harvested from the hypothalamus²⁴. Previously ICV cannulated, fasted, rats were administered either insulin (10 mU in 5 μ l delivered over 5 minutes, n=3) or an equal volume of aCSF (n=3). Thirty minutes later, brains were rapidly frozen in liquid nitrogen (<30 seconds after decapitation of anesthetized rats). The dissected hypothalamus was defined anatomically as posterior to the optic chiasm, anterior to the mammillary body, inferior to the thalamus, and $\pm$ 1mm lateral to the midline. Hypothalami were homogenized in RIPA buffer (1 % NP40, 0.5 % SDS, 0.1 mM PMSF), complete protease inhibitors cocktail (Roche, Indianapolis, IN) and phosphatase inhibitors: 1 mM active sodium orthovanadate and 1 mM NaF. Protein concentration was measured with the BCA protein quantification kit (Pierce, Rockford, IL). Protein extracts (100 μ g) were fractionated by electrophoresis on a 10% Bis-Tris Criterion XT (Biorad, Hercules, CA) gel and transferred to nitrocellulose membranes. Membranes were probed with the antibody against the phospho-AKT and total AKT (Cell Signaling Technology, Boston, MA). Primary antibody binding was detected by enhanced chemiluminescence ECL reagents (Perkin

Elmer, Wellesley, MA) on ISO-MAX films and quantified by ImageQuant software analysis (Amersham Pharmacia, Piscataway, NJ).

Analytical procedures

Plasma levels of glucose were measured by the glucose oxidase method (B-D Logic Glucometer). Epinephrine and norepinephrine analysis was measured with a single isotope derivative (radioenzymatic) method²⁸. Plasma corticosterone (MP Biomedicals, Orangeburg, NY) and glucagon (Linco Research, St. Charles, MO) were measured by radioimmunoassay. Plasma insulin was measured by ultrasensitive rat insulin ELISA (Crystal Chem Inc., Downers Grove, IL). In a few instances, values that were beyond the detectable range of an assay and could not be repeated were recorded, for statistical purposes, as the minimum or maximum detectable limit of that assay.

Statistical Analysis

Results are presented as the mean $\pm$ SEM. Statistical analysis was by two way ANOVA using SigmaStat 3.1 (Systat Software, San Jose, CA) or by an unpaired Student's t-test. Unless other stated, statistical significance was set at $P < 0.05$.

Results

The counterregulatory response to insulin-induced hypoglycemia was measured in rats receiving an intrahypothalamic (IH) or intracerebroventricular (ICV) infusion of either vehicle aCSF or insulin. In the basal state, weight, glucose, insulin, glucagon, corticosterone, epinephrine and norepinephrine levels were not significantly different between the groups. In response to the peripheral insulin infusion, hypoglycemia (40-50 mg/dl) was maintained with a co-infusion of glucose, although blood glucose levels trended down to a similar extent with both treatment groups (Figure 1A, 2A). During the hyperinsulinemic hypoglycemic clamp, insulin levels rose significantly with systemic insulin infusion and were not different between treatment groups (Figure 1B, 2B). With systemic insulin infusion, insulin levels rose 5-fold with low insulin doses (Figure 1B) and 50-fold with high insulin doses (Figure 2B). During the clamp, there were no significant differences between the glucose infusion rates between the aCSF and insulin infusion protocols, but as expected, the glucose infusion rates were significantly lower in response to low insulin doses ($5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (Figure 1C) than with high insulin doses ($15 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (Figure 2C). In response to hypoglycemia, the rate of glucose infusion was similarly matched with brain insulin or aCSF treatments. Epinephrine levels rose more than 60-fold (Figure 1D, 2D) and norepinephrine levels rose more than 4-fold (Figure 1E, 2E) without any significant differences between the aCSF and insulin treated groups. Likewise, glucagon increased more than 8-fold (Figure 1F, 2F) and corticosterone levels nearly doubled (Figure 1G, 2G) without any significant differences between the groups.

The counterregulatory response to a 2DG-induced glucoprivic signal was measured in rats receiving an intrahypothalamic infusion of either vehicle (aCSF) or insulin (15 mU over 5 hours). In the basal state, weight, glucose, insulin, glucagon, corticosterone, NE, and epinephrine were not significantly different between the two groups (intrahypothalamic aCSF vs. intrahypothalamic insulin). In response to 2DG, plasma glucose levels rose dramatically in both groups (Figure 3A), but there were no significant differences between the two groups. In response to 2DG and the subsequent rise in glycemia, insulin levels rose ~2-fold (Figure 3B) without any significant differences between the two groups. However, the increase in insulin levels achieved following 2DG was significantly less than the 5 fold increase in insulin levels that was achieved with the $5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infusion during the hyperinsulinemic clamp protocol (Figure 1B). Quantitatively similar counterregulatory responses were noted with 2DG administration as compared to insulin-induced hypoglycemia protocols. Specifically,

epinephrine levels rose more than 60-fold (Figure 3C), norepinephrine rose approximately 5-fold (Figure 3D), glucagon rose approximately 8-fold (Figure 3E), and corticosterone levels more than doubled (Figure 3F) without any significant differences between the intrahypothalamic insulin versus aCSF treatment groups.

In the absence of brain insulin infusion, CSF insulin concentrations were consistently $2.1 \pm 0.2\%$ of plasma insulin levels and were strongly correlated with plasma insulin levels ($R^2=0.82$, Figure 4A). In a separate group of rats, ICV administration of 10 mU of insulin increased AKT phosphorylation 5-fold ($P<0.05$), but not total AKT (not shown), as compared to aCSF treated control rats (Figure 4B).

Discussion

Studies have shown that the sympathoadrenal response to hypoglycemia may be enhanced⁶⁻⁹, not altered¹⁰⁻¹³, or inhibited¹⁴ by insulin. Animal studies have suggested that insulin might act in the cerebral circulation or in the brain to augment the sympathoadrenal response to hypoglycemia¹⁵⁻¹⁷. In this study, the acute infusion of insulin directly into either the third ventricle or directly into the mediobasal hypothalamus did not augment the sympathoadrenal response to insulin-induced hypoglycemia. These doses of insulin did reach high levels in the cerebrospinal fluid and these doses were effective in eliciting insulin signaling events. Similarly, in the absence of systemic insulin infusion, insulin infusion directly into the MBH does not augment the counterregulatory response to 2DG-induced glucopenia. Thus, under the conditions studied, acute brain insulin infusion did not augment the sympathoadrenal response to hypoglycemia nor to glucoprivation.

These studies were designed to optimize our ability to observe an effect of brain insulin infusion. Specifically, 1) the rats were fasted overnight to ensure low basal levels of insulinemia, 2) a relatively low dose of systemic insulin was administered to achieve hypoglycemia during the clamp in order to maximize our ability to detect a specific effect of intrahypothalamic insulin; 3) unlike milder degrees of hypoglycemia, a moderate depth of hypoglycemia (i.e., 40-50 mg/dl) was chosen as this depth of hypoglycemia was shown to more highly correlate epinephrine response to insulin levels²⁹; 4) to avoid the confounding effects associated with systemic insulin infusion, 2DG experiments were performed to elicit a glucoprivic counterregulatory response, 5) since the passage of insulin across the blood-brain-barrier (BBB) may be limited by a specific insulin transporter^{30;31}, the intracerebroventricular and intrahypothalamic insulin administration experimental approach was designed in order that insulin could bypass the BBB, 6) high pharmacological doses of intrahypothalamic and intracerebroventricular insulin were chosen to maximally elicit effects in the brain^{32;33} as they more closely correlate with augmentation of the sympathoadrenal response to hypoglycemia⁵, and 7) the MBH was specifically targeted for insulin infusion because nuclei within this region have been shown to be involved in mediating both brain insulin action^{34;35} as well as containing glucose sensing neurons that mediate the sympathoadrenal response to hypoglycemia^{18;19} 21.

The current set of experiments allowed for a comparison of the sympathoadrenal responses to high- and low-dose systemic insulin infusion clamp protocols by comparing the responses in the aCSF infused control rats. In the absence of brain insulin infusion and at matched degrees of hypoglycemia, low dose insulin infusion ($5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) increased systemic insulin levels 5-fold and resulted in a net epinephrine response (area under the curve) of $3.3 \pm 0.5 \times 10^5 \text{ pg} \cdot \text{min} \cdot \text{ml}^{-1}$. Higher dose insulin infusion ($15 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) increased systemic and CSF insulin levels 10-fold above low dose insulin infusion; yet at matched degrees of hypoglycemia, higher insulin doses yielded a net epinephrine response (area under the curve) similar to low dose insulin infusion ($3.9 \pm 0.4 \times 10^5 \text{ pg} \cdot \text{min} \cdot \text{ml}^{-1}$, $P=\text{NS}$). These findings indicate that even in

the absence of brain insulin infusion, marked increases in systemic insulin levels fail to increase the sympathoadrenal response to hypoglycemia in rats.

Davis, et al. demonstrated that infusion of insulin into the carotid and vertebral circulation of dogs augmented the sympathoadrenal response to hypoglycemia^{15;16}. There are many experimental variables that could have accounted for the notable differences between these previous results and our current study. Firstly, it is possible that a difference in species may account for the apparent discrepancy between the studies; although brain insulin actions seem to be more prominent in murine models than in the dog³⁶. Secondly, it is conceivable that insulin-induced changes in cerebral blood flow³⁷, rather than the direct actions of insulin in brain parenchyma, were responsible for the previously observed enhanced sympathoadrenal response to hypoglycemia with vertebral and carotid artery insulin infusion^{15;16}. Thirdly, insulin actions in the brain are not restricted to the hypothalamus. Areas in the hindbrain, particularly the nucleus of the tractus solitaries (NTS) are involved in the sensing of hypoglycemia and have been shown to be insulin-sensitive^{38;39}. Given the free distribution of insulin in the cerebrospinal fluid with ICV insulin administration, the absence of an effect on the counterregulatory response to hypoglycemia with ICV insulin experiments argues against any significant effect of insulin action in other brain areas, including the NTS. However, when considering possible brain regions affected, carotid and vertebral artery infusion would more likely engage a wider neuronal territory than the more focal delivery of insulin in the current studies, possibly accounting for the observed differences between studies. Finally, the effect of systemic hyperinsulinemia to augment the adrenomedullary response may be mediated by direct actions on the adrenal medulla⁴⁰. Since, however systemic (and presumably adrenal) insulin levels are matched during hyperinsulinemic hypoglycemic clamp experiments, adrenal insulin actions do not explain why head insulin infusion^{15;16} but not brain insulin infusion should alter the adrenomedullary response to hypoglycemia.

Since previous reports have shown that insulin entry into the CNS may be saturable at low levels of insulin^{30;31}, it could be argued that the peripheral insulin levels achieved during the hyperinsulinemic clamp could have elicited a maximal effect on brain insulin action, and theoretically could also have masked any additional effect of increased brain insulin infusion. There are several reasons to suggest that insulin's effect in the brain were not saturated. Firstly, our data did not show evidence for saturation, but rather a linear increase of CSF insulin concentration with increases in systemic insulinemia (Figure 4A). Secondly, many other studies indicate that the effect of insulin in augmenting the counterregulatory response to hypoglycemia is not saturated at low insulin levels but rather is enhanced with pharmacologic insulin infusions^{6;25-27}. Thirdly, a highly significant linear relationship between high plasma insulin levels and augmented epinephrine and norepinephrine responses to hypoglycemia⁵ supports the supposition that effect of insulin in the brain was not already maximal with the doses of insulin infusions used in these experiments. Finally, in the absence of a systemic insulin infusion, experiments performed using 2DG also demonstrated that brain insulin infusion did not augment counterregulation which supports the contention that insulin effects in the brain were not saturated by systemic insulin infusion during the hyperinsulinemic clamp experiments.

Although increased brain insulin did not increase the sympathoadrenal response, these experiments do not refute the notion that a small amount of insulin action in the brain may be critical for normal brain glucose sensing and the generation of a full sympathoadrenal response to hypoglycemia. Indeed, one of us (SJF) have previously demonstrated that the absence of insulin signaling in NIRKO mice causes a blunted sympathoadrenal response to insulin-induced hypoglycemia¹⁷. The defective sympathoadrenal response in the NIRKO mice appears to be specific to glucose sensing and may be related to changes in glucose transporter expression in the mediobasal hypothalamus²³. Consistent with the notion that a basal amount

of insulin is critical for normal brain glucose sensing, lowering basal insulin levels has been shown to decrease brain glucose uptake ⁴¹. Also, consistent with a role for basal hypothalamic insulin in modulating the counterregulatory response to hypoglycemia, acute blockade of insulin signaling in the ventromedial hypothalamus has recently been shown to augment the glucagon response to hypoglycemia ⁴².

Unlike the putative effect on the sympathoadrenal medullary response, the central effect of insulin in regulating the glucagon secretion is less well established. This study demonstrated that CNS insulin infusion did not affect the glucagon response (Figures 1F, 2F, 3E), although other studies report that insulin may act in the brain to increase ¹⁶, decrease ⁴², or not effect ¹⁷ the glucagon response to hypoglycemia.

As a marker of the hypothalamic-pituitary-adrenocortical response to hypoglycemia, the increased corticosterone levels were not significantly altered by brain insulin infusion in these experiments (Figures 1G, 2G, 3F). Insulin's effect on this hypothalamic-pituitary-adrenocortical response to hypoglycemia has also been equivocal with some studies indicating a significant stimulatory ^{6;8;25;26}, inhibitory ¹³, or no significant effect ^{7;9;11;12}.

In summary, under the conditions studied, it was found that neither intrahypothalamic nor intracerebroventricular insulin augmented the sympathoadrenal response or the response of other counterregulatory hormones, to insulin-induced hypoglycemia or to 2DG-induced glucoprivation in male Sprague-Dawley rats. Therefore, it is proposed that the previously noted acute actions of insulin to augment the sympathoadrenal response to hypoglycemia are likely mediated via insulin actions outside the central nervous system.

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Figure 1.

A-G. The counterregulatory response to a two hour, low dose ($5 \text{ mU.kg}^{-1}.\text{min}^{-1}$) hyperinsulinemic hypoglycemic clamp ($\sim 40\text{-}50 \text{ mg/dl}$) was measured in intrahypothalamic vehicle (aCSF) treated (solid circles) or intrahypothalamic insulin (15mU) treated (open circles) rats. Systemic metabolite and hormone measurements are shown at baseline and at 0, 30, 60, 90 and 120 minutes of hypoglycemia for A) glucose, B) insulin, C) glucose infusion rates, D) epinephrine, E) norepinephrine, F) glucagon, and G) corticosterone.

Figure 2.

A-G. The counterregulatory response to a two hour, high dose ($15 \text{ mU.kg}^{-1}.\text{min}^{-1}$) hyperinsulinemic hypoglycemic clamp ($\sim 40\text{-}50 \text{ mg/dl}$) was measured in intracerebroventricular vehicle (aCSF) treated (solid circles) or intracerebroventricular insulin (15mU) treated (open circles) rats. Systemic metabolite and hormone measurements are shown at baseline and at 0, 30, 60, 90 and 120 minutes of hypoglycemia for A) glucose, B) insulin, C) glucose infusion rates, D) epinephrine, E) norepinephrine, F) glucagon, and G) corticosterone.

Figure 3.

A-F. The glucoprivic response to 2-deoxyglucose (600 mg/kg IV bolus) was measured in intrahypothalamic vehicle (aCSF) treated (solid circles) or intrahypothalamic insulin (15mU) treated (open circles) rats. Systemic metabolite and hormone measurements are shown at baseline (time 0) and at 30, 60, 90 and 120 minutes following administration of 2-deoxyglucose for A) glucose, B) insulin, C) epinephrine, D) norepinephrine, E) glucagon, and F) corticosterone.

Figure 4.

A) In the absence of central nervous system insulin infusion, cerebrospinal fluid (CSF) insulin levels were highly correlated ($R^2=0.82$) with plasma insulin levels during the hyperinsulinemic hypoglycemic clamps. B) In a separate group of rats, ICV administration of 10 mU of insulin increased AKT phosphorylation 5-fold ($*p<0.05$) as compared to aCSF treated control rats.