Insulin Signaling in the Central Nervous System Is Critical for the Normal Sympathoadrenal Response to Hypoglycemia

Simon J. Fisher,[1] Jens C. Brüning,[2] Scott Lannon,[1] and C. Ronald Kahn[1]

Hypoglycemia, hypoglycemia unawareness, and impaired counterregulation are major challenges to the intensive management of type 1 diabetes. While the counterregulatory response to hypoglycemia is predominantly determined by the degree and duration of hypoglycemia, there is now evidence that insulin per se may influence the counterregulatory response to hypoglycemia. To define the role of insulin action in the central nervous system in regulating the counterregulatory response to hypoglycemia, mice with a brain/neuron-specific insulin receptor knockout (NIRKO) and littermate controls were subjected to 90-min hyperinsulinemic (20 mU · kg^{-1} · min^{-1}) -hypoglycemic (~1.5 mmol/l) clamps. In response to hypoglycemia, epinephrine levels rose 5.7-fold in controls but only 3.5-fold in NIRKO mice. Similarly, in response to hypoglycemia, norepinephrine levels rose threefold in controls, but this response was almost completely absent in NIRKO mice. In contrast, glucagon and corticosterone responses to hypoglycemia were similar in both groups. Thus, insulin action in the brain is critical for full activation of the sympathoadrenal response to hypoglycemia, and altered neural insulin signaling could contribute to defective glucose counterregulation in diabetes. *Diabetes* 54:1447–1451, 2005

For insulin-treated patients with diabetes, hypoglycemia is a major obstacle in their goals to normalize their blood glucose. A common finding in patients with type 1 diabetes is an impaired counterregulatory response to low blood glucose, which exacerbates the frequency and severity of insulin-induced hypoglycemic episodes (1). Decreased sympathoadrenal (norepinephrine and epinephrine) responses contribute to hypoglycemia unawareness in patients with type 1 diabetes by limiting the warning symptoms of tremulousness, palpitations, and anxiety (1). In coordinating the response to hypoglycemia, signals from central (2–4) and peripheral (5) glucose sensors are integrated in the brain to initiate the efferent autonomic responses. While the brain has classically been regarded as insulin insensitive, there has been clinical evidence to implicate that insulin can act in the central nervous system (CNS) to influence sympathetic nervous activity (6–8). Under conditions of hypoglycemia, when the autonomic nervous system is activated, elevated insulin levels have been shown to inhibit (9), augment (10–14), or not significantly effect (15–18) the sympathoadrenal response. Insulin may also alter the hypothalamic-pituitary-adrenal response as noted by an insulin-mediated augmentation of the cortisol response to hypoglycemia that was significant in some (10,11,14) but not all (12,13, 17,18) studies. Although the site of insulin action was not identified in these studies, it has been suggested that circulating insulin crosses the blood-brain barrier to directly activate the autonomic nervous system. A study (19) supporting this concept found that insulin infused into the carotid and vertebral arteries of dogs increases the autonomic response to hypoglycemia compared with animals receiving systemic insulin infusion.

In the present study, we have directly tested the role of the CNS insulin action in response to hypoglycemia using brain/neuronal insulin receptor knockout (NIRKO) mice created using Cre-lox technology (20). Our laboratory has shown that NIRKO mice have >95% reduction in the level of brain insulin receptor protein but no change in insulin receptor expression in skeletal muscle, heart, liver, kidney, spleen, and gonads. Inactivation of the brain insulin receptor has no impact on brain development or neuronal survival; however, it does result in a syndrome of mild diet-sensitive obesity and hypothalamic hypogonadism. We now show that the absence of CNS insulin receptors also limits the sympathoadrenal response to hypoglycemia, indicating that insulin signaling in the CNS is essential to generate a full counterregulatory response and that those alterations in brain/neural insulin action could contribute to more severe hypoglycemia in humans.

RESEARCH DESIGN AND METHODS

IR$^{(lox\text{-}lox)}$ mice homozygous for the floxed insulin receptor allele were bred with transgenic mice that express the Cre recombinase cDNA from the rat nestin promoter to generate (IR$^{(lox\text{-}lox)}$:nestin-Cre$^{(+/-)}$) neuronal/brain-specific insulin receptor knockout (NIRKO) mice (20). Genotypes were determined by PCR of tail DNA. All mice were housed on a 12-h light/dark cycle and fed a standard rodent diet (Mouse Diet 9F; PMI Nutrition International, St. Louis, MO) ad libitum. All procedures were in accordance with the Guide for the

From the [1]Research Division, Joslin Diabetes Center Department of Medicine, Harvard Medical School, Boston, Massachusetts; and the [2]Clinic for Internal Medicine and Center of Molecular Medicine, University of Cologne, Cologne, Germany.

Address correspondence and reprint requests to C. Ronald Kahn, MD, One Joslin Place, Room 705, Boston, MA 02215. E-mail: c.ronald.kahn@joslin.harvard.edu.

Received for publication 3 August 2004 and accepted in revised form 24 January 2005.

S.J.F. is currently affiliated with the Division of Endocrinology, Metabolism, and Lipid Research, Washington University School of Medicine, St. Louis, Missouri.

CNS, central nervous system.

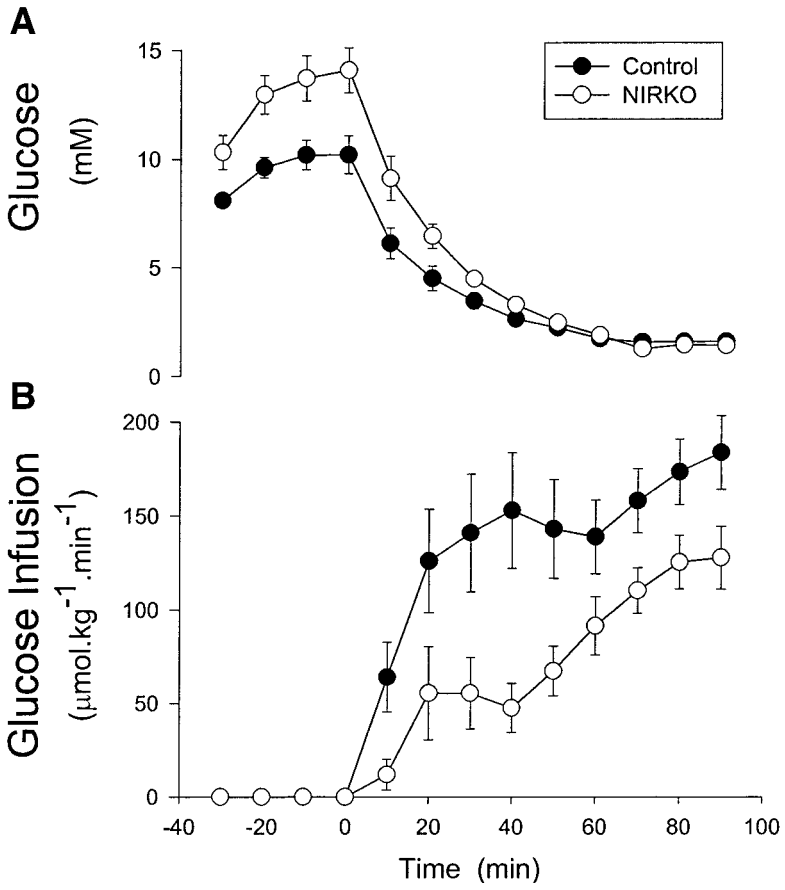

FIG. 1. Blood glucose levels and glucose infusion rates during the hypoglycemic-hyperinsulinemic clamp. After basal sampling at time −30, −20, −10, and 0 min, insulin was infused (200 mU/kg bolus and 20 mU · kg^{-1} · min^{-1} infusion) for the duration of the 90-min clamp. *A*: Blood glucose (from tail vein samples) was sampled at 10-min intervals and clamped at hypoglycemic levels (~1.5 mmol/l). *B*: Extreme care was taken with intravenous dextrose infusion to gradually lower glycemia and match groups for equivalent levels of hypoglycemia. Treatment groups were control (control, n = 11, ●) and NIRKO mice (n = 7, ○).

Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Joslin Diabetes Center animal care committee.

Hypoglycemic-hyperinsulinemic glucose clamps. Twelve-week-old male NIRKO (IR$^{(lox\text{-}lox)}$:nestin-Cre$^{(+/-)}$, n = 7) and littermate control (IR$^{(lox\text{-}lox)}$:nestin-Cre$^{(-/-)}$, n = 11) mice were used for these experiments. At this age, male NIRKO mice have indistinguishable body weights compared with controls (25.4 ± 1.3 vs. 24.3 ± 0.8 g, NS). Mice were anethesthetized with an intraperitoneal injection of 0.015 ml/kg of a 2.4% solution of 1:1 mixture of tert-amyl alcohol (Sigma, St. Louis, MO) and tribromoethanol (Sigma). After loss of pedal and corneal reflexes was assured, a catheter (MRE 025; Braintree Scientific, Braintree, MA) was inserted into the right internal jugular and advanced to the level of the superior vena cava. After 4–6 days of recovery, only mice that had regained >90% of their preoperative weight were studied. After a 5-h fast, awake mice were placed in a tail-restraint apparatus, and after an adjustment period, a 200-μl blood sample was collected from the tail tip. Separated plasma was stored for basal metabolite and hormone determinations while erythrocytes were resuspended in saline and reinfused to avoid volume depletion. After basal sampling, regular human insulin (Novo Nordisk, Clayton, NC) diluted in saline with 0.1% BSA (Sigma) was infused (200 mU/kg bolus and 20 mU · kg^{-1} · min^{-1}) at time zero. Blood samples (2.2 μl) were drawn at 10-min intervals to measure blood glucose (Glucose Analyzer; Bayer, Elkhart, IN). Extreme care was taken with the glucose infusion (50% dextrose; Abbott, Chicago, IL) to gradually lower glycemia and match groups for levels of hypoglycemia (~1.5 mmol/l). No seizure activity was noted. A 200-μl blood sample was drawn at the end of the 90-min hypoglycemic clamp for metabolite and hormone assays.

Assays. Radioimmunoassays were performed for glucagon (Linco Research, St. Charles, MO), corticosterone (ICN Biomedicals, Costa Mesa, CA), and epinephrine and norepinephrine (American Laboratory Products, Windham, NH).

Statistics. Results are presented as the mean ± SE. Statistical significance was set at $P < 0.05$ and determined by an unpaired Student's t test.

RESULTS

The counterregulatory response to hypoglycemia was studied during a 30-min basal period followed by a 90-min hypoglycemic-hyperinsulinemic clamp in male control and NIRKO mice. Glucose levels were slightly higher during the basal period in NIRKO mice compared with controls, reflecting the mild degree of insulin resistance in these mice (Fig. 1*A*). In response to insulin infusion, simultaneous dextrose infusion allowed for a gradual lowering of glycemia and matching groups for equivalent levels of hypoglycemia (control 1.6 ± 0.1, NIKRO 1.4 ± 0.1 mmol/l, NS). Glucose infusion rates were lower in the NIRKO than control mice (Fig. 1*B*), also consistent with a mild degree of insulin resistance.

Basal epinephrine levels were similar in control and NIRKO mice (5.2 ± 0.8 and 5.5 ± 0.7 nmol/l; Fig. 2*A*). During hypoglycemia, epinephrine levels in control mice rose 5.7-fold (to 30 ± 2 nmol/l). In NIRKO mice, epinephrine levels during hypoglycemia were significantly lower (20 ± 2 nmol/l), rising only 3.5-fold. Basal norepinephrine levels were similar in control and NIRKO mice (39 ± 8 and 48 ± 10 nmol/l, NS) (Fig. 2*B*). During hypoglycemia, norepinephrine levels rose threefold (to 121 ± 22 nmol/l) in the controls. Remarkably, norepinephrine levels during hypoglycemia in NIRKO mice (56 ± 12 nmol/l) were significantly lower than in control mice and did not differ from basal levels (NS vs. basal). Thus, the increment in norepinephrine during hypoglycemia was completely abrogated in NIRKO mice.

Glucagon levels were similar during the basal period (control 73 ± 4 and NIRKO 71 ± 10 ng/l) and rose threefold during hypoglycemia to a similar extent (control 237 ± 34 and NIRKO 220 ± 15 ng/l, NS) (Fig. 3*A*). Corticosterone levels were similar during the basal period (control 1.5 ± 0.2 and NIRKO 1.4 ± 0.4 μmol/l, NS) and

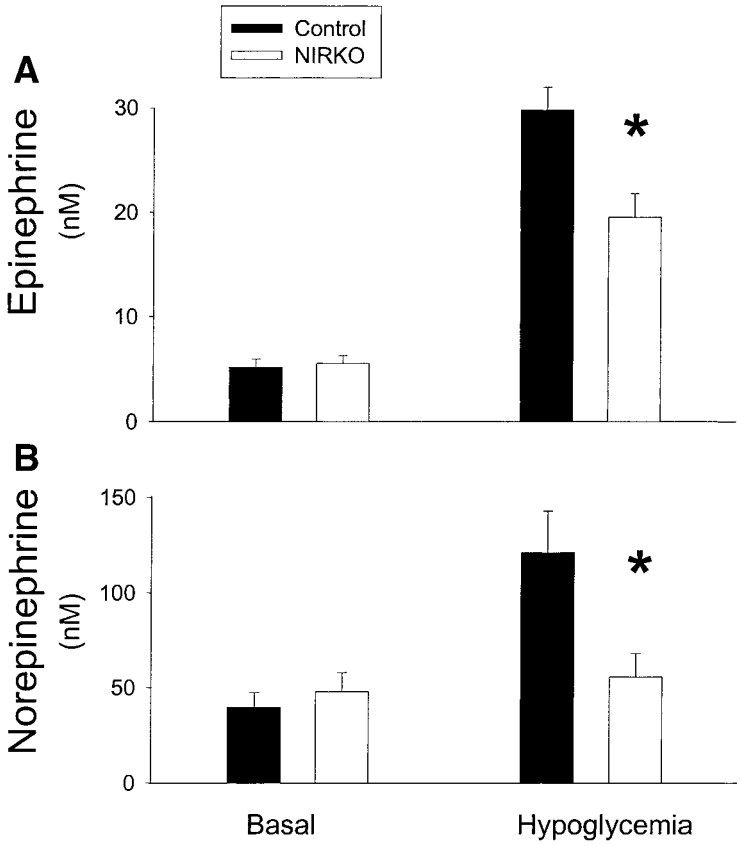

FIG. 2. Epinephrine and norepinephrine levels in the basal period and in response to hypoglycemia. *A*: In response to hypoglycemia, control epinephrine levels rose 5.7-fold. NIRKO epinephrine levels rose only 3.5-fold, demonstrating impaired adrenomedullary response to hypoglycemia ($P < 0.05$). *B*: In response to hypoglycemia, control norepinephrine levels rose threefold. There was no significant increase in NIRKO norepinephrine levels in response to hypoglycemia (NS vs. basal) indicating an abrogated norepinephrine response to hypoglycemia. Data are shown for control ($n = 11$, ■) and NIRKO ($n = 7$, □) mice. Statistical differences between study groups indicated by asterisks are discussed in RESULTS.

during hypoglycemia (control 1.6 ± 0.2 and NIRKO 1.7 ± 0.4 μmol/l, NS vs. basal) (Fig. 3*B*).

DISCUSSION

The role of insulin as an independent factor in the response to hypoglycemia has been debated. Elevated insulin levels have been shown to inhibit (9), augment (10–14), or not significantly effect (15–18) the sympathoadrenal response to hypoglycemia. In the present study, we demonstrate that insulin plays a role in protecting against hypoglycemia due, at least in part, to a direct action of insulin on the brain and/or nervous system. We show that the absence of insulin receptors in neuronal tissue limits the sympathoadrenal response to hypoglycemia (as noted by a ~50% reduced epinephrine response and a >90% reduced norepinephrine response).

The abrogation of the norepinephrine response in the absence of brain insulin signaling is consistent with other studies demonstrating that insulin, per se, can activate the sympathetic nervous system (6–8,21–23). The small fraction of norepinephrine that does enter the plasma is mostly derived from postganglionic sympathetic nerves terminals thus reflecting sympathetic neural activity under most conditions (24). Therefore, while the blunted NIRKO epinephrine response to hypoglycemia was due to an adrenomedullary defect, the absent NIRKO norepinephrine response to hypoglycemia indicates a specific defect in activation of the sympathetic nervous system. Based on studies in adrenalectomized patients, however, it has been suggested that under conditions of hypoglycemia, the norepinephrine response may be derived from the adrenal medulla (25). Therefore, we cannot rule out the possibility that the absent norepinephrine response is indicative of an adrenomedullary, rather than a specific sympathetic, nervous system defect. Whether this is because of the loss of insulin signaling in the brain or in sympathetic nerves is unclear; however, whatever the site, the effect is profound.

Our results are consistent with the hypothesis that acute increases in insulin cross the blood-brain barrier and augment counterregulation via acute interactions with CNS insulin receptors. These results in NIRKO mice, however, do not rule out the possibility that there may be chronic, generalized impairment in the activation of the sympathoadrenal system in response to stress, which may be mediated by CNS insulin receptors. Also, the observed defect in counterregulation may be absolute or relative. Perhaps more severe hypoglycemia could have elicited a full response. Similar phenomena have been observed in people with type 1 diabetes, whereby epinephrine responses can only be fully elicited at lower glucose concentrations (26). The depth of hypoglycemia has also been suggested to be an important factor in determining insulin's ability to amplify the sympathoadrenal response (27).

The regulation of glucagon secretion during hypoglycemia is complex. Glucagon levels rise in response to hypoglycemia, in spite of insulin's effect to limit α-cell glucagon release. It may be that systemic insulin delivery decreases intraislet insulin secretion to allow for increased glucagon response (28,29). Sympathetic or adrenal catecholamines also act to stimulate glucagon secretion during hypoglycema via adrenergic signaling. Under the current experimental conditions, however, the impaired catecholamine response to hypoglycemia did not diminish the full glucagon response in NIRKO mice, indicating that systemic catechol-

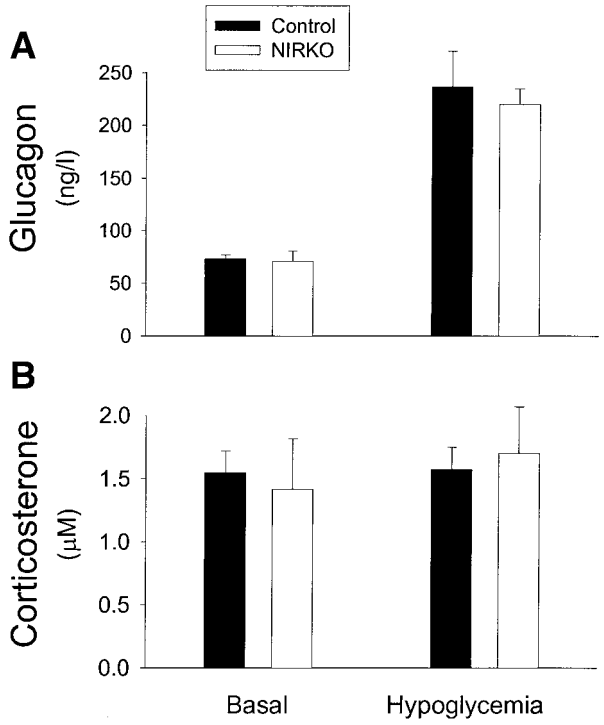

FIG. 3. Glucagon and corticosterone levels in the basal period and in response to hypoglycemia. *A*: Glucagon levels rose threefold in response to hypoglycemia in both control and NIRKO mice. *B*: Corticosterone levels were not different between treatment groups and did not increase in response to hypoglycemia. Data are shown for control (n = 11, ■) and NIRKO (n = 7, □) mice.

amines are qualitatively less important than other factors, such as local glycemia and perhaps intraislet insulin, in mediating the glucagon response to hypoglycemia.

The role of brain insulin receptor signaling in the corticosterone response is difficult to assess, since neither the control nor the NIRKO mouse demonstrated a corticosterone increase. It may be that elevated basal stress levels limited the corticosterone counterregulatory response.

The glucose infusion results must be interpreted with caution because during the first 50 min of the clamp, the higher glycemic levels in the NIRKO mice partially explain the lower rate of glucose infusion. At matched glycemia during the last 40-min period of the hypoglycemic clamp, however, the lower rate of glucose infusion in NIRKO does reflect whole-body insulin resistance (in the setting of a diminished counterregulation). In separate experiments under hyperinsulinemic-euglycemic clamp conditions, we (30) and others (31,32) have shown that insulin infusion rates of 20 mU · kg^{-1}· min^{-1} require glucose infusion rates of 580–900 μmol · kg^{-1} · min^{-1} to maintain euglycemia in control mice. In this current study, the same insulin doses (20 mU · kg^{-1} · min^{-1}) resulted in markedly reduced glucose infusion rates (~100–150 μmol · kg^{-1} · min^{-1}) indicating profound insulin resistance induced by hypoglycemic counterregulation. Results from some additional experiments conducted under euglycemic conditions indicate that the insulin resistance displayed in the NIRKO mice might be independent of the couterregulatory response. Low-dose (4 mU · kg^{-1} · min^{-1}) hyperinsulinemic-euglycemic (~7 mmol/l) clamp experiments, in which counterregulation would not be expected, demonstrated that NIRKO mice had whole-body insulin resistance located, and based on tracer studies, this was primarily located in the liver, not the muscle (33).

A chronic effect of CNS insulin signaling to mediate the brain's ability to sense and respond to ambient glycemia may be mediated via events downstream of insulin signaling, including glucose transport/phosphorylation and metabolism. We have shown that glucose uptake into the brain is decreased in NIRKO mice (33), although preliminary studies indicate no global change in expression of brain glucose transporters in NIRKO mice (S.J.F., C.R.K., unpublished results). While recurrent CNS neuroglucopenia leads to a specific adrenomedullary defect (hypoglycemia-associated autonomic failure) (1), we can only speculate as to whether a similar mechanism exists in the NIRKO mouse, whereby chronic decreased brain glucose uptake contributes to the blunting of the sympathoadrenal response to hypoglycemia.

In summary, the absence of brain insulin receptors limits a complete counterregulatory response to hypoglycemia, indicating that insulin action in the CNS is essential to generate a full sympathoadrenal response to low blood glucose. Since hypoglycemia and hypoglycemic unawareness are major problems and often rate limiting in the intensive management of type 1 diabetes, the role of insulin action in the brain needs to be considered as we develop new strategies to address these problems.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health to C.R.K. (DK 31036), grants from the Volkswagen Stiftung and the Köln Fortune program to J.C.B., and support from the Juvenile Diabetes Research Foundation to S.J.F.

REFERENCES

1. Cryer PE, Davis SN, Shamoon H: Hypoglycemia in diabetes. *Diabetes Care* 26:1902–1912, 2003
2. Biggers DW, Myers SR, Neal D, Stinson R, Cooper NB, Jaspan JB, Williams PE, Cherrington AD, Frizzell RT: Role of brain in counterregulation of insulin-induced hypoglycemia in dogs. *Diabetes* 38:7–16, 1989
3. Borg MA, Sherwin RS, Borg WP, Tamborlane WV, Shulman GI: Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. *J Clin Invest* 99:361–365, 1997
4. Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI: Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes* 44:180–184, 1995
5. Donovan CM, Hamilton-Wessler M, Halter JB, Bergman RN: Primacy of liver glucosensors in the sympathetic response to progressive hypoglycemia. *Proc Natl Acad Sci U S A* 91:2863–2867, 1994
6. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL: Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 87:2246–2252, 1991
7. Muscelli E, Emdin M, Natali A, Pratali L, Camastra S, Gastaldelli A, Baldi S, Carpeggiani C, Ferrannini E: Autonomic and hemodynamic responses to insulin in lean and obese humans. *J Clin Endocrinol Metab* 83:2084–2090, 1998
8. Paramore DS, Fanelli CG, Shah SD, Cryer PE: Forearm norepinephrine spillover during standing, hyperinsulinemia, and hypoglycemia. *Am J Physiol* 275:E872–E881, 1998
9. Diamond MP, Hallarman L, Starick-Zych K, Jones TW, Connolly-Howard M, Tamborlane WV, Sherwin RS: Suppression of counterregulatory hormone response to hypoglycemia by insulin per se. *J Clin Endocrinol Metab* 72:1388–1390, 1991
10. Davis SN, Dobbins R, Tarumi C, Colburn C, Neal D, Cherrington AD: Effects of differing insulin levels on response to equivalent hypoglycemia in conscious dogs. *Am J Physiol* 263:E688–E695, 1992
11. Davis SN, Goldstein RE, Jacobs J, Price L, Wolfe R, Cherrington AD: The

effects of differing insulin levels on the hormonal and metabolic response to equivalent hypoglycemia in normal humans. *Diabetes* 42:263–272, 1993
12. Davis SN, Shavers C, Collins L, Cherrington AD, Price L, Hedstrom C: Effects of physiological hyperinsulinemia on counterregulatory response to prolonged hypoglycemia in normal humans. *Am J Physiol* 267:E402–E410, 1994
13. Lingenfelser T, Overkamp D, Renn W, Buettner U, Kimmerle K, Schmalfuss A, Jakober B: Insulin-associated modulation of neuroendocrine counterregulation, hypoglycemia perception, and cerebral function in insulin-dependent diabetes mellitus: evidence for an intrinsic effect of insulin on the central nervous system. *J Clin Endocrinol Metab* 81:1197–1205, 1996
14. Davis MR, Mellman M, Shamoon H: Physiologic hyperinsulinemia enhances counterregulatory hormone responses to hypoglycemia in IDDM. *J Clin Endocrinol Metab* 76:1383–1385, 1993
15. Liu D, Moberg E, Kollind M, Lins PE, Adamson U: A high concentration of circulating insulin suppresses the glucagon response to hypoglycemia in normal man. *J Clin Endocrinol Metab* 73:1123–1128, 1991
16. Liu DT, Adamson UC, Lins PE, Kollind ME, Moberg EA, Andreasson K: Inhibitory effect of circulating insulin on glucagon secretion during hypoglycemia in type I diabetic patients. *Diabetes Care* 15:59–65, 1992
17. Mellman MJ, Davis MR, Shamoon H: Effect of physiological hyperinsulinemia on counterregulatory hormone responses during hypoglycemia in humans. *J Clin Endocrinol Metab* 75:1293–1297, 1992
18. Davis SN, Goldstein RE, Price L, Jacobs J, Cherrington AD: The effects of insulin on the counterregulatory response to equivalent hypoglycemia in patients with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 77:1300–1307, 1993
19. Davis SN, Colburn C, Dobbins R, Nadeau S, Neal D, Williams P, Cherrington AD: Evidence that the brain of the conscious dog is insulin sensitive. *J Clin Invest* 95:593–602, 1995
20. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. *Science* 289:2122–2125, 2000
21. Berne C, Fagius J, Pollare T, Hjemdahl P: The sympathetic response to euglycaemic hyperinsulinaemia: evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia* 35:873–879, 1992
22. Fruehwald-Schultes B, Kern W, Bong W, Wellhoener P, Kerner W, Born J, Fehm HL, Peters A: Supraphysiological hyperinsulinemia acutely increases hypothalamic-pituitary-adrenal secretory activity in humans. *J Clin Endocrinol Metab* 84:3041–3046, 1999
23. Schultz-Klarr S, Wright-Richey J, Dunbar JC: Plasma glucose, insulin and cardiovascular responses after intravenous intracerebroventricular injections of insulin, 2-deoxyglucose and glucose in rats. *Diabetes Res Clin Pract* 26:81–89, 1994
24. Shah SD, Tse TF, Clutter WE, Cryer PE: The human sympathochromaffin system. *Am J Physiol* 247:E380–E384, 1984
25. DeRosa M, Cryer P: Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation (Abstract). *Diabetes* 52 (Suppl. 1): A29, 2003
26. Dagogo-Jack SE, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus: recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia. *J Clin Invest* 91:819–828, 1993
27. Galassetti P, Davis SN: Effects of insulin per se on neuroendocrine and metabolic counter-regulatory responses to hypoglycaemia. *Clin Sci (Lond)* 99:351–362, 2000
28. Banarer S, McGregor VP, Cryer PE: Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. *Diabetes* 51:958–965, 2002
29. McCrimmon RJ, Evans ML, Jacob RJ, Fan X, Zhu Y, Shulman GI, Sherwin RS: AICAR and phlorizin reverse the hypoglycemia-specific defect in glucagon secretion in the diabetic BB rat. *Am J Physiol Endocrinol Metab* 283:E1076–E1083, 2003
30. Fisher SJ, Kahn CR: Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. *J Clin Invest* 111:463–468, 2003
31. Shen HQ, Zhu JS, Baron AD: Dose-response relationship of insulin to glucose fluxes in the awake and unrestrained mouse. *Metabolism* 48:965–970, 1999
32. Hazel M, Cooksey RC, Jones D, Parker G, Neidigh JL, Witherbee B, Gulve EA, McClain DA: Activation of the hexosamine signaling pathway in adipose tissue results in decreased serum adiponectin and skeletal muscle insulin resistance. *Endocrinology* 145:2118–2128, 2004
33. Fisher SJ, Bruning JC, Lannon S, Kahn CR: Impaired central nervous system insulin signaling decreases brain glucose uptake and leads to hepatic insulin resistance (Abstract). *Diabetes* 52 (Suppl. 1): A68, 2003