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

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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, noradrenaline 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-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-lox):nestin-Cre(lox-lox)) 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
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. 1A). 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, NIRKO 1.4 ± 0.1 mmol/l, NS). Glucose infusion rates were lower in the NIRKO than control mice (Fig. 1B), 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. 2A). 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 (30 ± 8 and 48 ± 10 nmol/l, NS) (Fig. 2B). 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. 3A). Corticosterone levels were similar during the basal period (control 1.5 ± 0.2 and NIRKO 1.4 ± 0.4 μmol/l, NS) and

**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 (n = 11, •) and NIRKO mice (n = 7, ○).
during hypoglycemia (control 1.6 ± 0.2 and NIRKO 1.7 ± 0.4 µmol/l, NS vs. basal) (Fig. 3B).

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 intraslet insulin secretion to allow for increased glucagon response (28,29). Sympathetic or adrenal catecholamines also act to stimulate glucagon secretion during hypoglycemia 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-
that NIRKO mice had whole-body insulin resistance lo-
counterregulation would not be expected, demonstrated
euglycemic (1450 DIABETES, VOL. 54, MAY 2005
rates of 20 mU (30) and others (31,32) have shown that insulin infusion
under hyperinsulinemic-euglycemic clamp conditions, we
diminished counterregulation). In separate experiments
reflect whole-body insulin resistance (in the setting of a
however, the lower rate of glucose infusion in NIRKO does
during the last 40-min period of the hypoglycemic clamp,
caution because during the first 50 min of the clamp, the
limited the corticosterone counterregulatory response.
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 corti-
costerone 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
insulin infusion rates (~100–150 μmol · kg⁻¹ · min⁻¹) 
indicating profound insulin resistance induced by hypogly-
cemic counterregulation. Results from some additional
experiments conducted under euglycemic conditions indi-
cate that the insulin resistance displayed in the NIRKO
mice might be independent of the counterregulatory re-
ponse. 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 lo-
cated, and based on further 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 signal-
ing, including glucose transport/phosphorylation and me-
tabolism. 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., unpub-
lished 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 re-
sponse to hypoglycemia.

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

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