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Combined corticotropin-releasing hormone and glucocorticoid deficiency does not enhance counterregulatory responses after recurrent hypoglycemia in mice⋆

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Abstract

Glucocorticoids and corticotropin-releasing hormone (CRH) have been proposed to inhibit counterregulatory responses to recurrent hypoglycemia. We used the CRH knockout (CRH KO) mouse to test the hypothesis that combined CRH and glucocorticoid deficiency would prevent development of counterregulatory deficits after repeated hypoglycemia. To develop a mouse model of recurrent hypoglycemia, we first tested the effects of daily lente insulin injection on counterregulatory responses to acute hypoglycemia in male C57BL/6 mice. Treatment with up to 250 U/kg per day lente insulin resulted in significantly greater decreases in plasma glucose, suggestive of impaired counterregulation, after hypoglycemia induced by acute insulin injection. Plasma catecholamine responses to hypoglycemia in repeatedly hypoglycemic C57BL/6 mice were unexpectedly higher than in naive mice, which we interpreted as a compensatory response to the greater decreases in plasma glucose. Lente insulin doses had to be reduced (50–75 U/kg per day) for CRH KO mice to survive repeated hypoglycemia. Wild-type (WT) mice treated with 50 to 75 U/kg per day lente insulin exhibited enhanced sympathetic activity after hypoglycemia, resembling the compensatory responses associated with impaired glucose homeostasis in C57BL/6 mice treated with 250 U/kg per day lente insulin. During acute hypoglycemia, CRH KO mice maintained higher plasma glucose levels that correlated with higher plasma norepinephrine and greater glycogen mobilization. Recurrent hypoglycemia did not enhance liver glycogen depletion or the markedly impaired glucocorticoid and epinephrine responses to hypoglycemia in CRH KO mice. Previously hypoglycemic CRH KO mice also did not display the further increases in sympathetic activity that in WT mice were suggestive of compensation for impaired counterregulation. Despite the apparent resistance of CRH KO mice to the counterregulatory effects of repeated hypoglycemia, their greater mortality after hypoglycemia tolerated by WT mice indicates that combined CRH and glucocorticoid deficiency does not significantly improve counterregulation after repeated hypoglycemia.

1. Introduction

Hypoglycemia unawareness is an increasingly common and potentially fatal complication of insulin use in type 1 diabetes mellitus (T1DM). Prior hypoglycemia blunts and lowers the glucose threshold for counterregulatory hormone and symptomatic responses to subsequent hypoglycemia, leaving patients with few physiological defenses or sensory signs to warn of dangerously low blood sugar [1]. Although avoidance of hypoglycemia can reverse many

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aspects of hypoglycemia unawareness in T1DM, hypoglycemia-induced glucagon secretion often remains impaired, particularly in long-standing T1DM. Inadequate glucagon secretion renders the sympathetic nervous system response to hypoglycemia more important because after glucagon, epinephrine has the most rapid action to restore blood sugar [1]. Therefore, identifying factors influencing sympathoadrenal responses to hypoglycemia will aid in preserving a key defense against hypoglycemia in T1DM.

As first demonstrated by Davis et al [2], prior increases in glucocorticoids can inhibit catecholamine as well as glucagon responses to additional hypoglycemic episodes. Such an effect would be of particular concern for hypoglycemia unawareness in longer-term T1DM, in which sympathoadrenal activity may have to compensate for defective glucagon responses. Davis laboratory findings have been confirmed by some [3,4], but not all [5–8], studies. Recently, data from Sherwin's laboratory have also implicated the main hypothalamic regulator of glucocorticoid secretion, corticotropin-releasing hormone (CRH), in suppressing sympathoadrenal activity evoked by hypoglycemia [6].

Inhibition of counterregulatory responses by the adrenocortical axis is puzzling, given that glucocorticoids are not only counterregulatory hormones themselves, but are also key regulators of the synthesis of epinephrine, another counterregulatory hormone [9]. To discriminate the influence of glucocorticoids on descending sympathetic vs local adrenal control of epinephrine synthesis, we studied a model of glucocorticoid deficiency, the CRH knockout (CRH KO) mouse. CRH KO mice have negligible glucocorticoid responses to most stressors, including acute hypoglycemia [10]. These mice display hallmarks of glucocorticoid deficiency, including elevated thymus weight and low body fat content [11–13], indicating that they have not compensated for chronically low glucocorticoid levels. CRH KO mice have relatively normal adrenomedullary morphology [11] and are capable of mounting detectable epinephrine responses to acute hypoglycemia. This knockout mouse is consequently a viable model to investigate the effects of glucocorticoids on adrenomedullary responses to recurrent hypoglycemia. We therefore hypothesized that (1) wild-type (WT) mice would exhibit impaired counterregulation after recurrent hypoglycemia, and (2) combined CRH and glucocorticoid deficiency would prevent CRH KO mice from developing counterregulatory deficits. To test these hypotheses, we have compared counterregulatory responses after repeated lente insulin–induced hypoglycemia or saline injection.

2. Research design and methods

2.1. Animals

All procedures were approved by the Institutional Animal Care and Use Committee of Albany Medical College. Mice were housed individually on a 12:12 light cycle and allowed free access to food and water except during the final hypoglycemia experiment. In experiment 1, male C57BL/6 mice (2–3 months old; Taconic Farms, Germantown, NY) were injected subcutaneously once per day with progressively higher doses of lente insulin (Lilly, Indianapolis, Ind) over 4 days, starting at 150 U/kg per day and finishing at 250 U/kg per day on the 2 days before challenge. This paradigm is a modification of a rat model of recurrent hypoglycemia [14,15]. Controls were injected with an equivalent volume of normal saline. Food intake and body weight were measured to the nearest 0.01 g on the day before the final hypoglycemic challenge. Mice were challenged within 2 hours of lights-on with insulin-induced hypoglycemia (8.5 U/kg regular insulin, SC). Mice were not fasted overnight, but were transferred to fresh cages without food immediately after insulin injection. Mice were bled by retro-orbital puncture at 0, 45, or 90 minutes after acute insulin injection. All blood samples were collected within 45 seconds of touching the cage to minimize handling stress effects on catecholamines and glucocorticoids. Plasma was separated and frozen at either −80°C (for catecholamines) or −20°C (for all other analytes) until assay.

For experiment 2, male WT and CRH KO mice were bred on a C57BL/6 × 129Sv background as previously described [11,12,16]. Litter- and age-matched WT and CRH KO mice were studied at 2 to 4 months of age. CRH KO mice did not survive the doses of lente insulin used in experiment 1, so repeated hypoglycemia was induced by injecting all mice subcutaneously with increasing lente insulin doses from 50 to 75 U/kg, once every 24 hours, for 5 days. Controls were injected with an equivalent volume of normal saline. Twenty-four hour food intake and body weight were measured to the nearest 0.01 g on the day before study. Mice were studied before and after a final, acute injection of regular insulin, as described above. Mice were bled by retro-orbital puncture and immediately decapitated 0 or 60 minutes after insulin injection. A piece of liver tissue was collected from each mouse at death and rapidly frozen for glycogen assay.

2.2. Plasma assays

Plasma glucose and corticosterone were measured by previously reported assays [10,17]. Plasma glucagon was measured using a kit from Linco (St Louis, Mo), halving all reagent and sample volumes. Assay performance was not altered by this modification. The interassay coefficient of variation of the glucagon assay was 8.4%. Plasma epinephrine, norepinephrine, and 3,4-dihydroxyphenylglycol (DHPG) were measured in extracted plasma by HPLC with electrochemical detection as previously described; extraction efficiency was monitored by spiking samples with 3,4-dihydroxybenzylamine [18].

2.3. Liver glycogen content

Liver glycogen content was assayed as reported by Lopez et al [19]. In brief, liver tissue was subjected to alkaline hydrolysis at 95°C in the presence of saturating amounts of sodium sulfate, followed by ethanol precipitation. The precipitated glycogen was dissolved in water and assayed spectrophotometrically at 490 nm against rabbit liver glycogen standards after sulfuric acid hydrolysis (Sigma, St Louis, Mo) and reaction with 5% phenol (American Bioanalytical, Natick, Mass).

2.4. Statistics

Data were analyzed by analysis of variance with post hoc testing by *t* test with Bonferroni correction for multiple comparisons. Significance was defined as $P < .05$. Data are presented throughout as mean ± SEM; where no error bars are visible, the scale of the symbol or the figure exceeded that of the error.

3. Results

3.1. Experiment 1: effects of repeated hypoglycemia on counterregulation in C57BL/6 mice

Treatment with increasing doses of lente insulin up to 250 U/kg produced sustained hypoglycemia in male C57BL/6 mice with free access to food. In a subset of mice, a single lente insulin injection transiently stimulated feeding (insulin vs saline injection, 1.37 ± 0.16 vs 0.29 ± 0.15 g per mouse over 6 hours; $P = .0012$; n = 4 and 6 per group, respectively), but maintained plasma glucose at 50 ± 8 mg/dL (n = 4) for up to 6 hours. Despite this hypoglycemia-induced feeding, repeated lente insulin injection did not alter total daily food intake or body weight (Table 1).

Although mice subjected to recurrent hypoglycemia had been hypoglycemic for at least 6 hours the day before, baseline glucose levels were similar to saline-injected controls on the day of study (Fig. 1, 0 minutes). However, plasma glucose fell to significantly lower levels at 45 and 90 minutes after acute hypoglycemic challenge in mice that had previously been hypoglycemic

(naive vs prior hypo: 45 minutes, 50 ± 2 vs 30 ± 6; 90 minutes, 33 ± 2 vs 12 ± 3; data repeated from Fig. 1 to clarify values potentially obscured by the scale of the graph).

At the time points studied, these lower glucose levels were not associated with lower counterregulatory hormone levels. Mice subjected to repeated hypoglycemia tended to have higher plasma epinephrine at 45 minutes ($P = .069$; Fig. 2A). Plasma norepinephrine was significantly higher at 45 minutes in this group and tended to be higher ($P = .092$) at 90 minutes (Fig. 2B). Hypoglycemia-induced levels of plasma corticosterone and glucagon were similar between mice, regardless of prior hypoglycemia exposure (Fig. 2C and D, respectively).

To determine if the higher hypoglycemia-induced norepinephrine levels in recurrently hypoglycemic mice were due to increased sympathetic nerve activity, we measured plasma levels of DHPG, a metabolite primarily reflecting neuronal norepinephrine turnover [20]. There were significant main effects of prior hypoglycemia, time, and the interaction of prior hypoglycemia with time on plasma DHPG responses to hypoglycemic challenge (Fig. 3). Plasma DHPG was significantly higher at all times in mice subjected to prior hypoglycemia (Fig. 3).

3.2. Experiment 2: effects of repeated hypoglycemia in WT and CRH KO mice

As noted in Research Design and Methods, lente insulin doses had to be reduced to allow survival of CRH KO mice during repeated hypoglycemia. In a subset of WT and CRH KO mice monitored during a single episode of lente insulin–induced hypoglycemia, 75 U/kg lente insulin produced equivalent hypoglycemia for up to 3 hours (47 ± 15 vs 47 ± 10 mg/dL, WT vs CRH KO mice; n = 3 per group), followed by a return to normal glucose levels within 5 to 8 hours that was indistinguishable between WT and CRH KO mice. Feeding during lente insulin–induced hypoglycemia was similar between genotypes (0.61 ± 0.24 vs 0.70 ± 0.09 g per mouse for every 3 hours, WT vs CRH KO, respectively; n = 3 per group). After repeated injection of lente insulin, total daily food intake was also comparable between genotypes (Table 2).

After daily injection of either lente insulin or saline, basal plasma glucose and liver glycogen content did not differ between genotypes or between naive and repeatedly hypoglycemic mice within each genotype (Fig. 4A and B, respectively). Because our initial experiments indicated that peak counterregulatory hormone responses were maintained between 45 and 90 minutes after insulin injection (Fig. 2 and unpublished observations), we selected a 60-minute time point for comparison of WT and CRH KO counter-regulatory responses. There was a significant main effect of time on plasma glucose and liver glycogen content (Fig. 4, initial vs acute hypoglycemia). In contrast to results in experiment 1, repeatedly hypoglycemia WT mice did not exhibit significantly lower plasma glucose than their naive counterparts after acute hypoglycemic challenge (Fig. 4C). Insulin-induced levels of plasma glucose also did not differ between naive and recurrently hypoglycemic CRH KO mice (Fig. 3C). However, there was a significant main effect of genotype on plasma glucose after hypoglycemia. Wild-type mice had lower plasma glucose levels than did CRH KO mice after both acute and repeated hypoglycemia, although these genotype differences were not significant by post hoc testing in either naive or repeatedly hypoglycemic mice (Fig. 4C).

Insulin-induced decreases in liver glycogen were significantly affected by both genotype and prior hypoglycemia (Fig. 4D). After hypoglycemia, liver glycogen content was significantly higher in naive WT mice than in previously hypoglycemic WT mice or in either CRH KO group (Fig. 4D). Hypoglycemia-induced liver glycogen levels in CRH KO mice were low and indistinguishable between naive and previously hypoglycemic mice (Fig. 4D).

Before acute hypoglycemic challenge, there was a significant effect of genotype on preinjection plasma epinephrine levels, with levels being significantly lower in CRH KO than in WT mice (Fig. 5A). Initial plasma levels of norepinephrine, corticosterone, and glucagon were similar between WT and CRH KO mice (Fig. 5B–D, respectively).

There was a significant main effect of time on all counterregulatory hormones (Fig. 5, initial vs acute hypoglycemia). Wild-type mice subjected to prior hypoglycemia tended to have higher hypoglycemia-induced plasma levels of all counterregulatory hormones than did their naive controls, although these differences were not significant (Fig. 5E–H). Wild-type mice injected acutely with subcutaneous saline did not exhibit significant increases in counter-regulatory hormones (not shown). Naive CRH KO mice exhibited significantly lower epinephrine, higher norepinephrine, and lower corticosterone after acute hypoglycemia than did naive WT mice. However, unlike WT mice, recurrently CRH KO mice did not show any tendency to increase counterregulatory hormones further after repeated exposure to hypoglycemia (Fig. 5E–H).

Both the trend toward greater hypoglycemia-induced epinephrine and norepinephrine in recurrently hypoglycemic WT mice and the significant norepinephrine responses to acute hypoglycemia in naive CRH KO mice suggested that sympathetic nerve activity was elevated in these groups. To evaluate this possibility, we also analyzed plasma DHPG [20]. There were significant main effects of genotype as well as time on plasma DHPG (Fig. 6). Baseline DHPG levels were significantly higher in naive CRH KO than in naive WT mice, but were not influenced by prior hypoglycemia (Fig. 6, initial). After hypoglycemic challenge, WT mice subjected to prior hypoglycemia had significantly higher plasma DHPG than did their naive counterparts (Fig. 6, acute hypoglycemia). As with plasma norepinephrine, naive CRH KO mice also exhibited significantly higher plasma DHPG after acute hypoglycemia than did naive WT mice, but recurrent hypoglycemia did not further increase DHPG levels in CRH KO mice (Fig. 6, acute hypoglycemia).

4. Discussion

We have shown that counterregulatory deficits can be produced in male mice by several episodes of prior hypoglycemia. After more severe repeated hypoglycemia, these deficits were primarily evident as greater insulin-induced decreases in plasma glucose; counterregulatory hormones were normal or (in the case of norepinephrine) elevated, most likely as a compensatory response to greater hypoglycemia. After less severe antecedent hypoglycemia, mice did not sustain greater hypoglycemia after acute insulin challenge, but still had elevated sympathetic activity resembling that associated with impaired glucose homeostasis after more severe prior hypoglycemia. CRH KO mice have markedly reduced glucocorticoid and epinephrine responses to repeated as well as acute hypoglycemia, but also appear insensitive to the effects of prior hypoglycemia on counterregulation.

Male C57BL/6 mice exposed to recurrent hypoglycemia in experiment 1 were less able to defend plasma glucose after insulin challenge. Lower glucose levels occurred in these mice despite higher plasma norepinephrine at 45 minutes and comparable levels of counterregulatory hormones at all other times relative to naive controls. Elevated norepinephrine responses in recurrently hypoglycemic mice were borne out by enhanced hypoglycemia-induced increases in DHPG, a metabolite closely linked to neuronal norepinephrine turnover [20]. Higher DHPG levels after hypoglycemia were not solely due to elevated baseline DHPG in previously hypoglycemic mice, since the significant analysis of variance interaction effect indicated that prior hypoglycemia enhanced the effects of acute hypoglycemia on plasma DHPG.

Although lower insulin-induced glucose levels in experiment 1 suggested that recurrent hypoglycemia impaired counterregulation, the unexpected preservation or enhancement of

counterregulatory hormone responses indicated that counterregulatory defects were incomplete. These results could be due to limitations of the experimental design or to the possibility that the mouse is not a good model for counterregulatory failure in humans. It is unlikely that prior hypoglycemia was inadequate to impair counterregulation because our model was comparable to previous approaches producing counterregulatory failure in rodents [14,15]. However, it is possible that greater insulin-induced decreases in glucose, caused by at least transient deficits in counterregulation, could have triggered compensatory hormone responses that obscured further evidence of counterregulatory impairment. We believe this explanation is more likely than the possibility that counterregulation in the mouse is not relevant to that in humans.

Glucose clamp techniques would have prevented uncontrolled decreases in plasma glucose that might stimulate, and therefore confound interpretation of, counterregulatory hormone responses. Glucose clamp studies of antecedent hypoglycemia in rats [7,14] have replicated the decreases in plasma catecholamines, glucagon, and glucocorticoids reported in humans [21], indicating that rodents are likely to be appropriate models of hypoglycemia-induced counterregulatory failure in humans. However, many glucose clamp studies showing evidence of counterregulatory failure in rats have also corroborated aspects of our results, in that some counterregulatory hormones are either not inhibited or are even stimulated [4,6–8]. We suspect that discrepancies between rodent and human models of counterregulatory failure reflect the difficulty of collecting frequent blood samples in rodents to match the timing of counterregulatory events. Common techniques for glucose clamps in mice, which involve either acute surgery, anesthesia, or tail nicks for blood collection [22,23], are also probably too stressful to evaluate catecholamines and glucocorticoids any more accurately than our current techniques. Thus, the limitations of the current experiments are no greater than those of other animal models.

In experiment 2, WT mice exposed to shorter episodes of antecedent hypoglycemia did not exhibit lower glucose levels after acute hypoglycemic challenge, but still tended to have higher levels of counterregulatory hormones. Although increments in individual hormones were not significant, their impact was indicated by significantly greater hypoglycemia-induced glycogen depletion in recurrently hypoglycemic vs naive WT mice. Significantly greater hypoglycemia-induced levels of DHPG also indicated that plasma norepinephrine was probably higher in previously hypoglycemic WT mice at earlier times after insulin injection. The enhanced DHPG response to hypoglycemia was similar to that associated with impaired defense of plasma glucose in previously hypoglycemic mice in experiment 1. This apparent compensatory sympathetic overactivation suggests that early stages of counterregulation were also impaired in WT mice in experiment 2, although we did not detect differences in plasma glucose.

CRH KO mice had higher plasma glucose levels relative to WT mice after both acute and repeated hypoglycemia. This result could be due either to lower insulin sensitivity or to greater secretion of and/or sensitivity to other counterregulatory factors in CRH KO mice. Insulin resistance seems unlikely. In keeping with their glucocorticoid deficiency, CRH KO mice exhibit characteristics of increased insulin sensitivity, including lower levels of plasma insulin and body fat [12,13].

Instead, greater glycogen mobilization after acute hypoglycemia suggests that counterregulatory hormone responses or actions were enhanced in CRH KO mice. Augmented hypoglycemia-induced glycogen depletion in naive CRH KO mice correlated most closely with evidence of enhanced sympathetic nerve activity, as indicated by elevated plasma levels of plasma norepinephrine and DHPG. Such elevations in sympathetic tone would be consistent with the effects of glucocorticoid deficiency [20]. Norepinephrine can directly stimulate hepatic glycogenolysis [24,25]; although it may be less effective than epinephrine in this regard

[25], it might have a more significant influence in CRH KO mice whose epinephrine secretion was substantially reduced. Greater glycogen depletion was not associated with higher glucagon levels in CRH KO mice, although it is also possible that CRH KO mice are more sensitive to the glycogenolytic actions of glucagon.

Unlike WT mice, CRH KO mice did not display further increases in counterregulatory hormones after recurrent hypoglycemia. As discussed above, the enhanced responses in WT mice, particularly for DHPG, are likely to reflect compensation for earlier deficits in counterregulation. The apparent lack of compensatory sympathetic overactivation, combined with the ability to maintain higher glucose levels during hypoglycemia, suggest that counterregulation is not impaired by prior hypoglycemia in CRH KO mice. Thus, CRH and glucocorticoid deficiency might, as hypothesized [2,4,6], protect against the counterregulatory deficits induced by repeated hypoglycemia.

However, this seeming resistance to the effects of prior hypoglycemia could also be ascribed to the possibility that counterregulatory responses are already maximal in CRH KO mice. Glycogen depletion and peripheral noradrenergic activity were pronounced in hypoglycemic CRH KO mice regardless of prior hypoglycemia and resembled the enhanced responses of recurrently hypoglycemic WT mice. The fact that CRH KO did not survive the more severe and prolonged hypoglycemia tolerated by WT mice indicates that even if CRH KO mice are capable of enhanced counterregulation, they have only a limited ability to sustain this counterregulatory effort.

CRH KO mice were vulnerable to hypoglycemia despite having relatively normal baseline levels of hepatic glycogen and plasma glucagon and elevated levels of plasma norepinephrine. Our available evidence suggests that this vulnerability is most readily attributable to glucocorticoid deficiency and to inadequate epinephrine synthesis secondary to glucocorticoid deficiency [9,26,27]. Although central CRH has also been suggested to be involved in sympathetic responses to hypoglycemia [28], findings in this regard have been contradictory [29]. Sympathoadrenal responses to other stimuli in CRH KO mice are also consistent with a primary effect of glucocorticoid rather than CRH deficiency on adrenomedullary regulation [27].

In light of a recent report that prior glucocorticoid exposure significantly enhanced hypoglycemic symptom awareness [5], our data collectively suggest that glucocorticoids may have a more complex role in defenses against hypoglycemia, including supportive as well as inhibitory effects. To the extent that epinephrine is a critical substitute for glucagon in T1DM patients at risk for hypoglycemia unawareness [1], potential inhibitory effects of glucocorticoids on sympathetic activity should be balanced against their role in maintaining adrenomedullary epinephrine synthesis [9].

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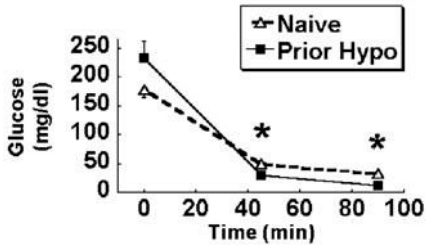

Fig. 1.

Plasma glucose before (0 minutes) or 45 and 90 minutes after acute hypoglycemic challenge in male C57BL/6 mice from experiment 1. Before challenge, mice were injected once per day with either saline (naive; open triangles and dashed lines) or lente insulin (prior hypo; black squares and solid lines), as described in Research design and methods. n = 3 per group for 0-minute measurements in both groups and 45-minute measurements in the prior hypo group; n = 4 in all other groups. *$P < .05$ vs naive mice.

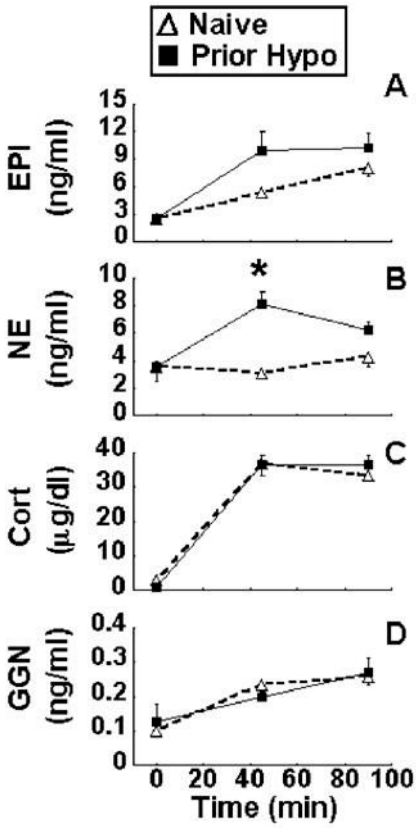

Fig. 2.

Plasma epinephrine (EPI; panel A), norepinephrine (NE; panel B), corticosterone (cort; panel C), and glucagon (GGN; panel D), either before (0 minutes) or 45 or 90 minutes after acute hypoglycemic challenge in male C57BL/6 mice from experiment 1. Groups and symbols are as described in Fig. 1. *$P < .05$ vs naive mice.

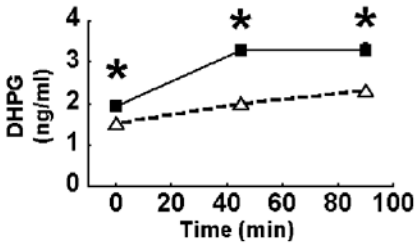

Fig. 3.

Plasma levels of the norepinephrine metabolite DHPG, either before (0 minutes) or 45 or 90 minutes after acute hypoglycemic challenge in male C57BL/6 mice from experiment 1. Groups and symbols are as described in Fig. 1. **P* < .05 vs naive mice.

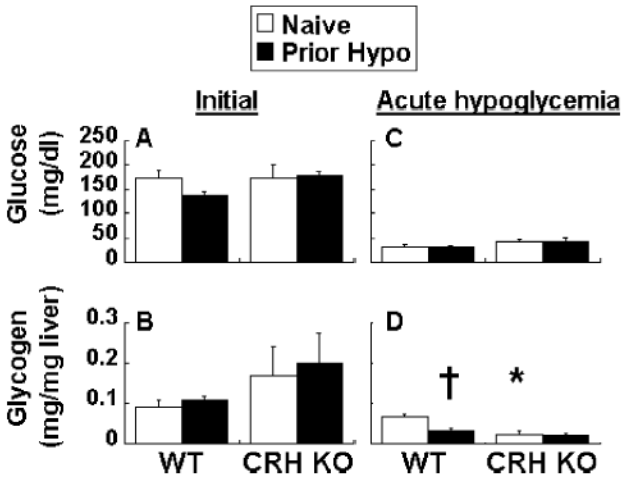

Fig. 4.

Plasma glucose (A, C) and liver glycogen content (B, D) in WT and CRH KO mice from experiment 2, either before (initial; panels A and B) or 60 minutes after acute challenge with insulin-induced hypoglycemia (acute hypoglycemia; panels C and D). Mice were subjected to daily injection of saline (naive; white bars) or lente insulin (prior hypo; black bars) before the study, as described in Research design and methods. Group sizes for data depicted in panels A and B (initial) were 4 (WT, naive), 3 (WT, prior hypo), 6 (CRH KO, naive), and 5 (CRH KO, prior hypo). Group sizes for data depicted in panels C and D (acute hypoglycemia) were 6 (WT, naive), 13 (WT, prior hypo), 11 (CRH KO, naive), and 4 (CRH KO, prior hypo). *$P < .05$ vs WT in same chronic treatment group. $^{\dagger}P < .05$ vs naive mice in same genotype.

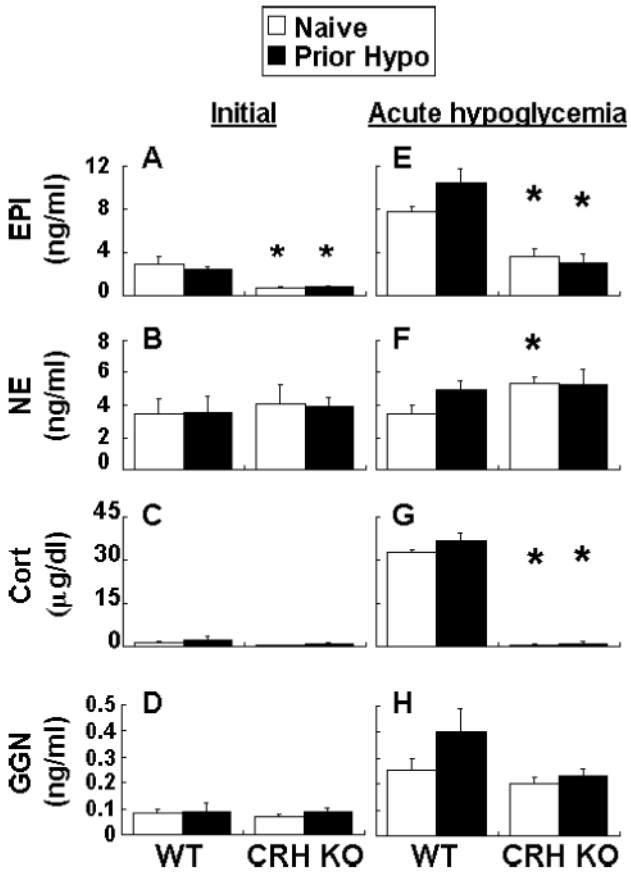

Fig. 5.

Plasma epinephrine (EPI; panels A, E), norepinephrine (NE; panels B, F), corticosterone (cort; panels C, G), and glucagon (GGN; panels D, H) levels in WT and CRH KO mice from experiment 2, either before (initial; A–D) or 60 minutes after acute challenge with insulin-induced hypoglycemia (acute hypoglycemia; E–H). Groups and symbols are as described in Fig. 4. *$P < .05$ vs WT in same chronic treatment group.

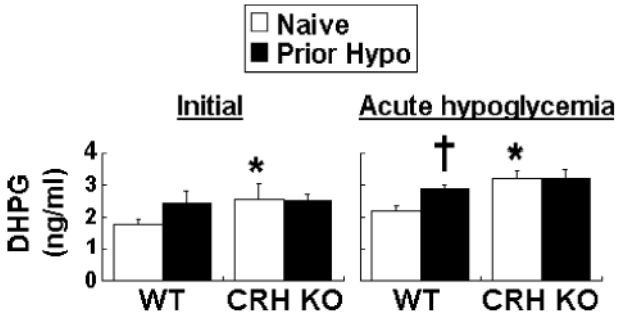

Fig. 6.

Plasma DHPG in WT and CRH KO mice from experiment 2, either before (initial; left) or 60 minutes after acute challenge with insulin-induced hypoglycemia (acute hypoglycemia; right). Groups and symbols are as described in Fig. 4. *$P < .05$ vs WT in same chronic treatment group. $^{\dagger}P < .05$ vs naive mice in same genotype.

Table 1

Daily (24-hour) food intake and body weight in C57BL/6 mice subjected to repeated saline injection (naive; n = 11) or lente insulin–induced hypoglycemia (prior hypo; n = 10) in experiment 1

	Naive	Prior hypo
Body weight (g)	24.69 ± 0.40	25.56 ± 0.45
24-h food intake (g per mouse)	4.17 ± 0.14	4.86 ± 0.41

Measurements were made to the nearest 0.01 g on the day before the final hypoglycemic challenge.

Table 2

Daily food intake (g per mouse per 24 hours) in WT and CRH KO mice after repeated saline injection (naive) or lente insulin–induced hypoglycemia (prior hypo) in experiment 2

Genotype	Naive	Prior hypo
WT	4.55 ± 0.24 (n = 10)	5.06 ± 0.19 (n = 16)
CRH KO	5.28 ± 0.23 (n = 17)	5.08 ± 0.43 (n = 8)

Food intake was measured to the nearest 0.01 g over the 24 hours preceding the final hypoglycemic challenge. One food measurement in the CRH KO, prior hypo group was lost because of spillage.