AICAR and phlorizin reverse the hypoglycemia-specific defect in glucagon secretion in the diabetic BB rat

R. J. McCrimmon, M. L. Evans, R. J. Jacob, X. Fan, Y. Zhu, G. I. Shulman, and R. S. Sherwin. AICAR and phlorizin reverse the hypoglycemia-specific defect in glucagon secretion in the diabetic BB rat. Am J Physiol Endocrinol Metab 283: E1076–E1083, 2002. First published July 30, 2002; 10.1152/ajpendo.00195.2002.—Individuals with type 1 diabetes demonstrate a hypoglycemia-specific defect in glucagon secretion. To determine whether intraislet hyperinsulinemia plays a role in the genesis of this defect, glucagon-secretory responses to moderate hypoglycemia induced by either insulin or a novel combination of the noninsulin glucose-lowering agents 5-aminoimidazole-4-carboxamide (AICAR) and phlorizin were compared in diabetic BB rats (an animal model of type 1 diabetes) and nondiabetic BB rats. The phlorizin-AICAR combination was able to induce moderate and equivalent hypoglycemia in both diabetic and nondiabetic BB rats in the absence of marked hyperinsulinemia. Diabetic BB rats demonstrated impaired glucagon and epinephrine responses during insulin-induced hypoglycemia compared with nondiabetic rats. In contrast, both glucagon (9- to 10-fold increase) and epinephrine (5- to 6-fold increase) responses were markedly improved during phlorizin-AICAR hypoglycemia. Combining phlorizin, AICAR, and insulin attenuated the glucagon response to hypoglycemia by 70% in the diabetic BB rat. Phlorizin plus AICAR had no effect on glucagon secretion in response to hypoglycemia in type 1 diabetes. Possible mechanisms include 1) a generalized impairment of the α-cell to external stimuli, 2) an impaired α-cell response to autonomic stimuli, 3) impaired activation of autonomic inputs, 4) a specific defect in the α-cells’ ability to recognize hypoglycemia, and 5) a direct, or indirect, suppressive effect of exogenous insulin on the α-cell (49, 50). The fact that the glucagon-secretory defect is selective (glucagon-secretory responses to other stimuli remaining largely intact), develops concomitantly with progressive loss of β-cell function (21), and is not affected by pharmacological blockade of classical autonomic mediators (27, 53) and the fact that isolated α-cells do not appear able to respond to hypoglycemia per se (40) suggest an abnormality in signaling to the α-cell in which insulin, directly or indirectly, may play a role. This proposal is not new and was first raised by Samols et al. (45). A number of studies have examined the effects of insulin on both glucagon and adrenomedullary responses to hypoglycemia (1, 2, 10–16, 18–20, 30–36, 39), the results of which have proven inconclu-

THE RISK OF LONG-TERM COMPLICATIONS of diabetes can be reduced by aggressive insulin therapy to lower blood glucose levels in type 1 diabetes (51). Unfortunately, the global implementation of such intensive treatment regimens is limited by an increased risk of mild and severe hypoglycemia (52). This phenomenon is, in part, a consequence of the nonphysiological nature of current replacement therapies, which will occasionally result in a relative excess of insulin delivery. However, the risk of hypoglycemia is further increased in type 1 diabetes by defects in the neurohumoral responses that would otherwise defend against a low blood glucose. In nondiabetic subjects, release of the counterregulatory hormone glucagon and the catecholamines, epinephrine and norepinephrine, in response to early hypoglycemia, is a potent stimulus to help rapid restoration of normal blood glucose levels. Most patients with type 1 diabetes develop defective glucagon responses to hypoglycemia within several years of disease onset (22), and some diabetic subjects develop additional counterregulatory deficiencies, displaying abnormal catecholamine responses to a falling blood glucose. The combination of abnormal glucagon and catecholamine responses to hypoglycemia markedly increases the risk of severe hypoglycemia (8).

Because the α-cell is subject to both local pancreatic and distal (neural and circulating humoral) influences, it has proven extremely difficult to determine the principal mechanistic defect that is responsible for the impaired glucagon-secretory response to acute hypoglycemia in type 1 diabetes. Possible mechanisms include 1) a generalized impairment of the α-cell to external stimuli, 2) an impaired α-cell response to autonomic stimuli, 3) impaired activation of autonomic inputs, 4) a specific defect in the α-cells’ ability to recognize hypoglycemia, and 5) a direct, or indirect, suppressive effect of exogenous insulin on the α-cell (49, 50). The fact that the glucagon-secretory defect is selective (glucagon-secretory responses to other stimuli remaining largely intact), develops concomitantly with progressive loss of β-cell function (21), and is not affected by pharmacological blockade of classical autonomic mediators (27, 53) and the fact that isolated α-cells do not appear able to respond to hypoglycemia per se (40) suggest an abnormality in signaling to the α-cell in which insulin, directly or indirectly, may play a role. This proposal is not new and was first raised by Samols et al. (45). A number of studies have examined the effects of insulin on both glucagon and adrenomedullary responses to hypoglycemia (1, 2, 10–16, 18–20, 30–36, 39), the results of which have proven inconclu-

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sive. These studies have been limited by the inability to induce significant hypoglycemia without the use of high concentrations of insulin and, in the case of animal studies, by the choice of animal model used. In this study, we present evidence that, by inducing hypoinsulinemic hypoglycemia in the diabetic BB rat, a validated animal model of type 1 diabetes that shares the counterregulatory defects of its human counterpart (28, 37, 38, 41), we are able to significantly improve both the glucagon- and catecholamine-secretory responses to hypoglycemia.

EXPERIMENTAL PROCEDURES

Animals

Diabetic BB/Wor and nondiabetic BB/Wor rats were purchased from Biotech Research Models (Rutland, MA). The diabetic BB rats were all insulin dependent at the time of purchase (14–28 days duration) and maintained on PZI insulin (once daily sc injection at 1700), with doses based on body weight, and a daily measurement of tail vein glucose at 0900. Animals were housed in the Yale Animal Care Facility, kept in an environmentally controlled room with a 12:12-h light-dark cycle, and fed a standard ad libitum rat chow diet that contained 22% protein, 5% fat, and 51% carbohydrate (Agway Prolab 3000, Syracuse, NY). The protocol was reviewed and approved by the Yale Animal Care and Use Committee.

Surgery

Six to ten days before being studied, both diabetic (mean weight 268 g, range 215–329) and nondiabetic (mean weight 249 g, range 190–326) BB animals underwent aseptic surgery under ketamine (60 mg/kg im) and pentobarbital (21 mg/kg ip) anesthesia. Long-term catheters were implanted in the aortic arch, via the jugular vein, as previously described (6). Catheters were flushed with heparin (42 U/ml) and polyvinylpyrrolidone (1.7 g/ml) solution, tunneled subcutaneously, and externalized behind the head through a skin incision. Only animals that recovered completely and showed no signs of infection postoperatively were used.

Infusion protocols

Diabetic BB rats received a reduced dose of insulin (60% of daily requirement) the evening before being studied. All animals were studied nonfasted while awake and moving freely about their cages. On the morning of the study (0800), catheters were opened, and a primed infusion of insulin (20 mU·kg⁻¹·min⁻¹ Humulin R; Eli Lilly, Indianapolis, IN) was started. A 10% dextrose infusion was simultaneously infused and adjusted according to plasma glucose measurements taken at 10-min intervals to achieve the desired glucose level. Diabetic BB rats received additional saline (0.9%) to help compensate for any dehydration resulting from nocturnal hyperglycemia. During this first period of the experiment, the aim was to achieve and maintain the plasma glucose at euaglycemia [100 mg/dl (5.5 mM)]. Insulin infusions ran for 120–180 min in both diabetic and nondiabetic groups to allow for the diabetic BB animals, which started the morning with higher plasma glucose levels, to fall gradually to euaglycemia. After 60 min of controlled hyperinsulinemic euaglycemia, one of four separate groups of experiments was conducted.

Insulin-induced hypoglycemia (protocol 1). In diabetic (n = 6) and nondiabetic (n = 6) BB rats, the insulin infusion was continued at the same rate (20 mU·kg⁻¹·min⁻¹), but the dextrose infusion rate was reduced to allow plasma glucose to fall to ~2.8 mM (50 mg/dl), where it was then maintained.

Phlorizin-AICAR-induced hypoglycemia (protocol 2). In diabetic (n = 10) and nondiabetic (n = 5) BB rats, phlorizin was given as a subcutaneous bolus (0.5 g/kg body wt) at the commencement of the study. Insulin (20 mU·kg⁻¹·min⁻¹) and 10% dextrose were then infused to maintain hyperinsulinemic euaglycemia, as detailed in protocol 1. After this baseline period, the insulin infusion was stopped, and after a delay of 15 min (during which time the dextrose infusion was continued to maintain euaglycemia), an infusion of 5-aminoimidazole-4-carboxamide (AICAR) was started (80 mg/kg body wt given over 2 min, followed by a continuous infusion of 10 mg·kg⁻¹·min⁻¹). Plasma glucose was allowed to fall to ~2.8 mM (50 mg/dl), where it was then maintained by use of a variable infusion of 10% dextrose.

Phlorizin is an inhibitor of renal glucose reabsorption and is often used to attenuate hyperglycemia in diabetic animals but cannot by itself induce significant hypoglycemia. We found that combining phlorizin with AICAR (an agent that lowers glucose by stimulating muscle glucose uptake and decreasing hepatic glucose production), we were able to induce moderate and sustained hyperglycemia even in insulin-dependent diabetic BB rats. The doses of phlorizin and AICAR and timing of infusions were based on early pilot studies.

Insulin-phlorizin-AICAR-induced hypoglycemia (protocol 3). In diabetic (n = 8) and nondiabetic (n = 5) BB rats, all three agents were used to induce hypoglycemia in these animals. As with protocol 2, animals received a bolus of phlorizin before the start of the insulin and dextrose infusions. After the period of hyperinsulinemic euaglycemia, the insulin infusion was continued, and an additional primed infusion of AICAR (as in protocol 2) was started. Again, the glucose was allowed to fall to ~2.8 mM (50 mg/dl), where it was then maintained by use of a variable infusion of 10% dextrose.

Phlorizin-AICAR euglycemia (protocol 4). As in protocol 2, diabetic (n = 5) animals received an initial bolus of phlorizin and were then started on the same insulin and dextrose infusions. At the end of the baseline period, the insulin infusion was stopped, and after a delay of 15 min during which time the dextrose infusion was continued, a primed infusion of AICAR (as in protocol 2) was started. Plasma glucose was maintained at euaglycemia throughout by use of a variable infusion of 10% dextrose.

Baseline blood was sampled twice for later measurement of glucagon, epinephrine, norepinephrine, and insulin (20 min before and at the end of the hyperinsulinemic euaglycemic phase). Sampling was repeated after 30 and 60 min of hypoglycemia. In protocol 4 (phlorizin-AICAR euglycemia), hormonal samples were taken at 30 and 60 min after the start of the AICAR infusion. Blood obtained from littermates before the study was transfused during the experiments to quantitatively replace blood withdrawn for hormone sampling.

Analytical Methods and Calculations

Plasma glucose was measured in duplicate using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma free insulin was measured using a rat insulin radioimmunoassay (Linco Research, St. Charles, MO). Plasma glucagon was measured by radioimmunoassay using an antibody spe-
cific to pancreatic glucagon (Linco Research). Plasma catecholamines were measured by HPLC.

**Statistics**

Data from diabetic and nondiabetic BB rats were first analyzed separately. The means of the two baseline and the two hypoglycemia measures from each study were used (a separate analysis of individual responses at 30 and 60 min of hypoglycemia did not differ from the overall analysis). Hormonal results in the text are displayed as the incremental increase above baseline during hypoglycemia, unless otherwise stated. Results (except for catecholamines) were analyzed using a repeated-measures ANOVA with subsequent post hoc testing by Scheffé-Bonferroni testing and results expressed as means ± SE. Plasma catecholamines were not normally distributed, so the nonparametric Kruskall-Wallis test was used, and results are presented as medians (interquartile range). A P value < 0.05 was considered statistically significant.

**RESULTS**

**Hypoglycemic Studies**

**Insulin and glucose.** We were able to achieve very similar hypoglycemic challenges in all treatment groups regardless of the agents used to lower blood glucose in both nondiabetic (Fig. 1A) and diabetic (Fig. 1B) groups. As expected, circulating insulin levels rose significantly in both diabetic (peak insulin 4,273 ± 1,042 pmol/l) and nondiabetic rats (3,175 ± 720 pmol/l) during the insulin hypoglycemia studies. In contrast, plasma free insulin fell markedly when hypoglycemia was induced by phlorizin-AICAR in both diabetic (348 ± 168 pmol/l) and nondiabetic (286 ± 152 pmol/l) groups (Fig. 2). When insulin was combined with phlorizin-AICAR, plasma insulin levels rose to the same degree as during the insulin hypoglycemia studies [5,375 ± 788 and 5,546 ± 875 pmol/l in nondiabetic and diabetic rats, respectively, both P = not significant (NS) vs. insulin hypoglycemia].

**Glucagon.** Baseline glucagon levels were similar in all animal groups (41 ± 7, 53 ± 5, 38 ± 10 pg/ml during insulin, phlorizin-AICAR, and phlorizin-AICAR-insulin studies in nondiabetic BB rats, respectively, and 54 ± 3, 70 ± 10, 57 ± 2 pg/ml during insulin, phlorizin-AICAR, and phlorizin-AICAR-insulin studies in diabetic BB rats, respectively). Glucagon levels rose significantly in the nondiabetic rats during insulin-induced hypoglycemia (221 ± 50 pg/ml; P < 0.05), whereas insulin hypoglycemia in diabetic BB rats produced only a small and insignificant rise in plasma glucagon (63 ± 10 pg/ml). In contrast, hypoglycemia induced by the phlorizin-AICAR combination stimulated a marked rise in plasma glucagon in both diabetic (349 ± 80 pg/ml) and nondiabetic (559 ± 70 pg/ml) rats (Fig. 3). However, although the magnitude of the glucagon-secretory response to phlorizin-AICAR hypoglycemia was greater in the nondiabetic BB rats compared with baseline, the relative change in the glucagon-secretory response compared with insulin hypoglycemia was greater in the diabetic BB rats. As shown in Fig. 3, when insulin and phlorizin-AICAR were then combined to induce hypoglycemia, there was a marked reversal of the glucagon-stimulatory effect in diabetic BB rats (peak glucagon 124 ± 15 pg/ml; P < 0.05 vs. phlorizin-AICAR hypoglycemia). In contrast to the effect on diabetic animals, the addition of insulin to phlorizin-AICAR had no suppressive effect on glucagon responses in nondiabetic BB rats (peak glucagon 579 ± 95 pg/ml).

**Epinephrine responses.** In nondiabetic BB rat groups, epinephrine rose briskly during hypoglycemia induced by either insulin [median (interquartile range) incremental increase 6.3 (3.8–8.4) ng/ml] or phlorizin-AICAR [2.8 (2.0–4.4) ng/ml], with no statistically significant differences between treatment groups (Fig. 4A). In contrast, diabetic BB rats had markedly lower epinephrine responses to insulin-induced hypoglycemia [incremental increase 0.7 (0.2–1.6) ng/ml] compared with the nondiabetic rats (P < 0.05). However, during phlorizin-AICAR [3.8 (1.4–5.3) ng/ml], the epinephrine response in diabetic rats was indistinguishable from that in nondiabetic animals (P = NS vs. nondiabetics). As a result, the increment in epinephrine responses in the diabetic rats was five- to sixfold higher than during insulin hypoglycemia, although this difference just failed to reach statistical signifi-
cance ($P = 0.06$ vs. insulin hypoglycemia). Epinephrine responses remained brisk when insulin was combined with phlorizin-AICAR epinephrine responses in both diabetic and nondiabetic groups [incremental increases $2.4$ (3.3–4.4) and $3.3$ (2.5–3.4) ng/ml, respectively; both $P = \text{NS}$ vs. phlorizin-AICAR hypoglycemia].

Norepinephrine responses. As shown in Fig. 4

Euglycemic Studies

The euglycemic studies employing phlorizin-AICAR were performed only in diabetic animals. As with our hypoglycemic studies, plasma free insulin fell markedly (Fig. 2). There were, however, no significant changes in glucagon [$36.9 \pm 7.6$ pg/ml during baseline and $47 \pm 22$ pg/ml during phlorizin-AICAR euglycemia], epinephrine [increment $0.1$ (0.1–0.2) ng/ml; Fig. 4A], or norepinephrine [increment $0.2$ (0.1–0.5); Fig. 4B], a markedly different outcome from that seen when hypoglycemia was allowed to occur.

DISCUSSION

In this study, we set out to examine whether the glucagon-counterregulatory response to hypoglycemia in diabetes might be altered if a novel noninsulin agent lowered the blood glucose. We demonstrated that the failure of the $\alpha$-cell to secrete glucagon during hypoglycemia in the spontaneously diabetic BB rat was not evident if hypoglycemia was induced in the presence of very low circulating levels of insulin. This is the first demonstration of a substantial glucagon response to a hypoglycemic agent in an animal model of type 1 diabetes.

We used a protocol in which hypoglycemia was induced during insulin withdrawal by means of a combination of phlorizin and AICAR in the insulin-deficient diabetic BB rat. AICAR is an adenosine analog, which is taken up by cells and then phosphorylated to AICA.
ribose monophosphate, which mimics the effect of AMP to stimulate AMP-activated protein kinase (AMPK). AMPK is a ubiquitous enzyme found in many organ systems including the brain (17), pancreas (42), and skeletal muscle (3) and is postulated to play a pivotal role in gauging intracellular fuel stores (23). Activation of AMPK by AICAR lowers blood glucose by stimulating skeletal muscle glucose uptake (3) and decreasing hepatic glucose output (55). Phlorizin, an inhibitor of renal glucose reabsorption, was used to facilitate hypoglycemia.

Crucial to the interpretation of studies in this field is the animal model chosen to examine the glucagon-secretory response to hypoglycemia. In these studies, we have used the diabetic BB rat, because, like its human counterpart, it develops a spontaneous autoimmune insulin-dependent diabetes (37, 38). Moreover, the diabetic BB rat, like individuals with type 1 diabetes, demonstrates a stimulus-specific defect in the glucagon response to insulin-induced hypoglycemia, with glucagon secretion to arginine remaining intact (41). This defect appears a few days after the loss of insulin-producing β-cells and is followed by a later, milder impairment in epinephrine release during hypoglycemia (28, 41). The diabetic BB rat also allows the study of counterregulatory defects in animals free of the potentially confounding toxic effects inherent in the use of alloxan or streptozotocin (29). Moreover, alloxan and streptozotocin will usually induce a state of stable hyperglycemia indicating a relative, but not an absolute, insulin-deficient state that may be an important factor in the genesis of the α-cell-secretory defect. Our present results, with diabetic BB rats demonstrating markedly attenuated glucagon and subnormal catecholamine responses to insulin-induced hypoglycemia compared with their nondiabetic counterparts, are consistent with previous data (28, 41) and with studies in human type 1 diabetic subjects (5, 22); this agreement supports the validity of our model.

The underlying cause of the loss of glucagon secretion during hypoglycemia in type 1 diabetes is uncertain, particularly as the diabetic α-cell is still able to release glucagon in response to other stimuli such as amino acids (42). Possible causative roles for hyperglycemia, autonomic dysfunction, and/or hyperinsulinemia have been suggested (48, 50, 54). The suppressive effect of insulin on glucagon secretion was first recognized over 30 years ago and was postulated to play a role in the physiological regulation of glucagon secretion from α-cells (1, 35, 44, 45). Because of the unique microarchitecture of pancreatic islets (44), blood flows from the β-cell-rich islet core out into the α-cell-rich periphery, exposing α-cells to extremely high concentrations of insulin that are capable of suppressing glucagon secretion at normal blood glucose levels (49). In nondiabetic subjects, endogenous insulin is suppressed if blood glucose starts to fall. This glucose-sensing system has recently been shown to be surprisingly sensitive to even small reductions in blood glucose (20). Thus absolute levels of insulin bathing α-cells fall dramatically, even though circulating systemic insulin levels will be elevated if exogenous insulin is used to induce hypoglycemia. It follows from the intraislet insulin hypothesis that the fall in local ambient insulin levels will release α-cells from the effects of tonic inhibition by insulin and facilitate the activation of glucagon secretion. The situation is different in type 1 diabetes, where selective autoimmune destruction of the β-cell means that there is no endogenous insulin secretion, so that β-cells are exposed to exogenous insulin only. This means that, during insulin-induced hypoglycemia, intraislet insulin increases in the type 1 diabetic pancreas, suggesting that the failure of intraislet insulin to fall is a key factor in the genesis of this defect. However, it is also recognized that some individuals with type 1 diabetes and residual C-peptide secretion show defective glucagon secretion to hypoglycemia. Additionally, it has been reported recently in type 2 diabetes of long duration that the glucagon-secretory response to insulin-induced hypoglycemia is markedly reduced (46). Although these individuals had lower fasting C-peptide levels (1.1 ± 0.4 ng/ml) compared with those type 2 diabetic individuals who showed intact glucagon counterregulation, C-peptide levels were still higher than one might expect to find in some of those individuals with early type 1 diabetes and “normal” glucagon counterregulation (46). This raises the possibility that other mechanisms may contribute to defective glucagon counterregulation in both type 1 and type 2 diabetes.

There is good evidence that insulin inhibits glucagon secretion in both animals and humans. Perfusion of isolated pancreata in nondiabetic rats with insulin antibodies stimulated glucagon secretion at moderate [4.45 mM (80 mg/dl)] hypoglycemia (35, 44). Hyperinsulinemic euglycemia attenuates the glucagon-secretory response to arginine in humans (1), and higher insulin infusion rates, compared with lower insulin infusion rates, have been reported to blunt the glucagon response to a falling blood glucose in some (18, 33, 34), but not all, trials (13, 15). In addition, intravenous tolbutamide (a sulfonurea that stimulates insulin secretion through a direct action on the β-cell) has recently been shown to almost completely suppress the glucagon-counterregulatory response to hypoglycemia in nondiabetic human subjects (2).

Many of these studies have used high insulin levels or intravenous tolbutamide, where it is conceivable that the impaired glucagon secretion may have been affected by intraislet hyperinsulinemia per se rather than having resulted from a failure of insulin to fall during hypoglycemia. Our results provide strong supportive evidence that the abnormal glucagon responses to insulin-induced hypoglycemia in the diabetic BB rat arise in large part because of a failure of plasma insulin to fall during insulin hypoglycemia.

In our study, when insulin was used in combination with phlorizin-AICAR to induce hypoglycemia, glucagon results were again, to a large extent, attenuated. However, the fact that combining insulin with AICAR and phlorizin did not completely reverse the augmented glucagon response implies an independent ef-
fect of either AICAR or phlorizin. This effect would appear to be specific to the hypoglycemic state, because the phlorizin-AICAR combination did not induce glucagon secretion under euglycemic conditions. It is conceivable that AICAR itself might alter α-cell secretion directly through actions on adenosine A1-type receptors, or nitric oxide synthase (7). In pancreatic β-cell lines, AMPK activity and insulin secretion fluctuate inversely in response to changes in blood glucose (43). However, AICAR had little effect on AMPK or insulin secretion in these cell lines and was even reported to paradoxically stimulate insulin release from isolated pancreatic islets at a low glucose concentration (43). Furthermore, our euglycemic studies in the diabetic BB rat suggest that, at the doses we used, there was no significant direct effect of AICAR or phlorizin to stimulate glucagon secretion. Another possibility is that the improved glucagon response in diabetic rats during hypoinsulinemic hypoglycemia resulted in part from the magnified catecholamine response. However, during the insulin-phlorizin-AICAR studies, we observed brisk catecholamine-secretory responses, whereas glucagon responses in the diabetic rats were diminished. Taken together, these results suggest that the effects that we observed were predominantly a consequence of altered circulating insulin levels; however, an insulin-independent effect of phlorizin-AICAR may have contributed as well.

It was noticeable that phlorizin-AICAR hypoglycemia in nondiabetic BB rats evoked a greater absolute rise in glucagon compared with diabetic BB rats. Additionally, replacement of insulin (insulin combined with phlorizin and AICAR) in the diabetic animals, but not in the nondiabetic animals, resuppressed the glucagon response to hypoglycemia. Why might this have happened? The most likely explanation is that the relative change in intras islet insulin is very different in animals with and without endogenous insulin secretion under the different study conditions. Additionally, the different animal types may show different sensitivities to the suppressive effect of insulin and/or the potential stimulatory effect of phlorizin-AICAR. Further studies using different concentrations of insulin and/or AICAR would be required to more fully address this issue.

We also demonstrated that lowering blood glucose with phlorizin-AICAR augmented the subnormal catecholamine responses to hypoglycemia in the diabetic BB rat. However, in contrast to glucagon responses, epinephrine and norepinephrine secretion in the diabetic rats remained brisk during the insulin-phlorizin-AICAR studies. Phlorizin-AICAR had no stimulatory effect on the already brisk responses observed to insulin-induced hypoglycemia in nondiabetic animals, whereas norepinephrine responses appeared to be enhanced. These results raise the possibility that the phlorizin-AICAR combination was having a direct stimulatory effect on the adrenomedullary response to hypoglycemia. The mechanism by which it might do so is not clear. The predominant hypoglycemia sensor(s) that initiates and controls counterregulatory responses to a fall in blood glucose is thought to be located within the brain (4), most likely in the hypothalamus (6). The enzyme AMPK is found in the brain, although at lower levels than in other organ systems (17). It is, therefore, possible that AICAR may have had a direct, central stimulatory effect on brain centers, triggering adrenomedullary responses.

It is also conceivable that AICAR may have actions on peripheral hypoglycemia sensors such as those postulated in the liver or portal system (25, 26). Finally, the possibility should also be considered that unrecognized antecedent hypoglycemia contributed to the subnormal epinephrine response seen in the diabetic BB rats. In diabetic BB rats (41) and in human subjects (24), antecedent hypoglycemia is known to suppress the counterregulatory response to subsequent hypoglycemia. This is a functional defect, referred to as hypoglycemia-associated autonomic failure (HAAF) (9). Although steps were taken to reduce the likelihood of antecedent hypoglycemia occurring through a reduction in the insulin dose given on the day before the study, we cannot exclude the possibility that this may have contributed in some way to our findings. However, it is not clear why HAAF had no effect on the epinephrine response to phlorizin-AICAR hypoglycemia. Overall, the results from this study appear to indicate a potential effect of phlorizin-AICAR on the sympathoadrenal response to acute hypoglycemia that is independent of insulin. Better understanding of the mechanisms mediating the restoration of catecholamine responses to phlorizin-AICAR hypoglycemia in diabetic models might advance our understanding of how defects in catecholamine defenses against a low glucose develop in type 1 diabetes.

In keeping with the present observations in spontaneously diabetic rats, it has been suggested that the long-term use of twice daily phlorizin therapy might preserve counterregulatory responses in streptozotocin-induced diabetic rats (47). In that study, phlorizin produced a partial improvement in glucagon responses to insulin-induced hypoglycemia compared with untreated or insulin pellet-treated diabetic animals. That protocol, unlike ours, examined the chronic effects of noninsulin treatment in preventing the development of abnormal counterregulation and demonstrated an effect on glucagon secretion only during hypoglycemia. In contrast, we have demonstrated marked improvements of both glucagon and adrenomedullary responses during acute hypoglycemia; i.e., no period of adaptation was required before responses were restored. Nevertheless, taken together, that study and ours suggest that novel treatment protocols of diabetes with noninsulin alternative agents might allow preservation of normal counterregulation or restoration of abnormal counterregulatory responses to normal. In turn, this might allow more aggressive blood glucose management with less risk of hypoglycemia.

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