Differential Impairment of Glucagon Responses to Hypoglycemia, Neuroglycopenia, Arginine, and Carbachol in Alloxan-Diabetic Mice

Bo Ahrén, Gerald J. Taborsky Jr, and Peter J. Havel

To gain insight into the mechanisms responsible for the loss of the glucagon response to insulin-induced hypoglycemia in type 1 diabetes, glucagon responses to 4 different stimuli were examined over 3 months of diabetes in alloxan-treated mice. At 1, 6, and 12 weeks after alloxan (60 mg/kg), phloridzin (0.1 g/kg) was administered to overnight fasted diabetic mice to match the glucose levels of those in nondiabetic control mice before administration of the acute stimuli. Despite the elevation of baseline glucagon levels produced by the phloridzin treatment, the glucagon responses to insulin (2 U/kg intraperitoneally [IP])-induced hypoglycemia was not impaired at 1 week. However, the response was reduced by greater than 60% after 6 and 12 weeks of diabetes ($P < .05$). In contrast, the glucagon response to arginine (0.25 g/kg intravenously [IV]) was not reduced after 1, 6, or 12 weeks of diabetes, ruling out a generalized impairment of the A-cell responses. The glucagon response to the neuroglucopenic agent, 2-deoxyglucose (2-DG; 500 mg/kg IV) was impaired, like that to insulin-induced hypoglycemia, after 6 and 12 weeks of diabetes ($P < .05$), suggesting that supersensitivity to the potential inhibitory effects of exogenous insulin is not the mechanism responsible for the impairment. Finally, the glucagon response to the cholinergic agonist, carbachol (0.53 μmol/kg IV), was not impaired in the diabetic animals, arguing against a defect in the A-cell's responsiveness to autonomic stimulation. The data suggest that the impairment of the glucagon response to insulin-induced hypoglycemia in alloxan diabetic mice is not due to a generalized impairment of A-cell responsiveness, to desensitization by a suppressive action of insulin, or to impairment of the A-cell response to autonomic stimuli. The remaining mechanisms which are likely to explain the late loss of the glucagon response to insulin-induced hypoglycemia include (1) a defect in the A-cell recognition of glucopenic stimuli, or (2) a defect in the autonomic inputs to the A cell that are known to be activated by glucopenic stimuli.
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IT IS WELL ESTABLISHED that increased glucagon secretion during acute hypoglycemia is an important factor for the restoration of euglycemia.[1,2] It is also established that the glucagon response to insulin-induced hypoglycemia is impaired in diabetes.[3-8] This impairment is of clinical importance, since defective glucagon secretion increases the risk of severe hypoglycemia during insulin treatment of type 1 diabetes.[1] The glucagon response to insulin-induced hypoglycemia is also impaired in rodents with experimentally induced diabetes, although the impairment occurs more rapidly than in humans.[9-14]

Several mechanisms have been proposed to explain the impaired glucagon response to insulin-induced hypoglycemia in type 1 diabetes.[15] These possible mechanisms include (1) a generalized impairment of A-cell responses to external stimuli; (2) a supersensitivity of the A cell to the suppressive effects of exogenous insulin[16,17]; (3) an impairment of the A-cell responsiveness to autonomic stimuli; (4) impaired activation of the autonomic inputs to the pancreas[15]; and (5) a specific defect in the ability of the A cell[5] or the islet[18] to recognize and respond to glucopenic stimuli.

Although thoroughly testing each possible mechanism is a Herculean task, it is possible to gain significant insight into the plausibility of certain mechanisms by examining the glucagon response to different stimuli over time after the onset of diabetes. Therefore, we conducted such a study examining the glucagon response to 4 different stimuli in alloxan-diabetic mice at different time points after induction of insulin-deficient diabetes. A dose of alloxan was employed (60 mg/kg intravenously [IV]) which induces a stable and permanent diabetes that does not require insulin treatment to prevent ketoacidosis.[19] This latter point makes it unlikely that any impairment of the glucagon responses observed would be due to hypoglycemia-associated autonomic failure (HAAF), because such prior hypoglycemia usually requires administration of exogenous insulin.[20,21] Another important factor in the study design was that the glycemia was normalized in the diabetic mice on the day of each study, before administration of any of the 4 glucagon stimuli. Thus, the variability in the baseline glucose levels that could potentially confound the comparisons of the glucagon responses between the diabetic and nondiabetic mice was eliminated. The treatment used to acutely reverse the diabetic hyperglycemia was phloridzin. Phloridzin reduces circulating glucose through renal excretion by an insulin-independent mechanism[22] and has been used previously to normalize glucose levels in rodent models of insulin-independent diabetes.[23]

It was first important to establish that an impairment of the glucagon response to insulin-induced hypoglycemia actually develops in this model of diabetes. To that end, the glucagon response to insulin-induced hypoglycemia was examined after 1, 6, and 12 weeks of alloxan-induced diabetes. To determine if any observed impairment in the glucagon response to insulin-induced hypoglycemia was due to a generalized impairment of the A cell, the glucagon response to IV administration of the amino acid, L-arginine, was next examined. To determine if supersensitivity to the suppressive effect of exogenous insulin

on the A cells might be responsible for the impairment of the glucagon response to insulin-induced hypoglycemia, the glucagon response to another glucopenic stimulus, not associated with markedly increased insulin levels, was examined. This alternative glucopenic stimulus was the glucose analog, 2-deoxyglucose (2-DG), which produces intracellular glucopenia by competing with glucose for membrane transport and intracellular phosphorylation.[24] The resulting central neuroglucopenia activates the autonomic nervous system, thereby increasing glucagon seretion, without lowering circulating glucose concentrations or, in alloxan-induced diabetes, increasing the exposure of the A cell to insulin. Finally, to determine if the responsiveness of the A cell to cholinergic stimulation might be impaired, we examined the glucagon response to the cholinergic agonist, carbachol.

MATERIALS AND METHODS

Animals and Study Design

Female mice of the NMRI strain were obtained from Bomholtgaard Breeding and Research Centre, Ry, Denmark, at 4 weeks of age. The animals were fed a standard pellet diet and tapwater ad libitum. One week after arrival, ie, at the age of 5 weeks, animals were injected IV with alloxan monohydrate (Sigma Chemical Co, St Louis, MO; 60 mg/kg); controls were injected with saline. The experiments were performed at 1, 6, and 12 weeks later in conscious animals. Each animal therefore underwent 3 experiments. The animals were not treated with insulin during the 12 weeks of the study. The study was approved by the Animal Ethics Committee at Lund University.

Experiments

Following an overnight fast, a blood sample was rapidly obtained from the intraorbital, retrobulbar plexus at 8 AM. Thereafter, the diabetic animals were injected intraperitoneally (IP) with phloridzin (Sigma; 0.1 g/kg; dissolved in 10% dimethyl-sulfoxide [DMSO]); controls were given an equal volume of DMSO. The injections were repeated at 9 AM, 10 AM, and 11 AM. At noon, ie, 4 hours after the first phloridzin/vehicle injection, a new blood sample was taken and the mice were injected with either human insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark; 2 U/kg IP), the amino acid, L-arginine hydrochloride (Sigma; 0.25 g/kg IV), the glucose analog, 2-DG (Sigma; 0.5 g/kg IV), or the cholinergic agonist, carbachol (Sigma; 0.53 μmol/kg IV). New blood samples were then taken after 15, 30, 45, and 60 minutes (insulin experiments) or after 2 minutes (2-DG, arginine, and carbachol experiments). The doses of these agents and the 2-minute time point for sampling after administration of 2-DG, arginine, or carbachol were selected to achieve a rapid glucagon response, as shown in previous experiments[25-28] (Ahrén, unpublished data). After blood sampling, plasma was immediately separated following a centrifugation at 4°C and stored at −20°C until analysis.

Analysis

Plasma glucagon was determined radioimmunochemically with the use of guinea pig antiglucagon antibodies specific for pancreatic glucagon, ^{125}I-labeled glucagon as tracer, and glucagon standard (Linco Research, St Charles, MO). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 7.5 pg/mL and the coefficient of variation is less than 9%. The antibody is specific for pancreatic glucagon and exhibits cross-reactivity with gut-derived glucagon molecules of less than 0.1% (Linco). Plasma insulin was determined by radioimmunoassay with the use of a guinea pig anti-rat insulin antibody, ^{125}I-labeled porcine insulin as tracer, and rat insulin as standard (Linco). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). Plasma glucose concentrations were determined with the glucose oxidase technique.

Calculations and Statistics

Means ± SEM are shown. The area under the curve (AUC) above baseline glucagon levels from 0 to 60 minutes after insulin administration was calculated by the trapezoid method. Statistical analyses were performed with the SPSS for Windows system (SPSS, Inc, Chicago, IL). Statistical comparisons for the change in glucagon levels in each experiment between alloxan-diabetic and control mice were performed by the use of Student's unpaired t test with Bonferroni's correction for multiple comparisons.

RESULTS

Baseline Values

Fasting plasma glucose and insulin levels before the first administration of phloridzin or vehicle and after 4 hours of phloridzin/vehicle treatment are shown in Table 1. Phloridzin treatment reduced circulating glucose levels in alloxan-diabetic mice, resulting in glucose levels similar to those in the nondiabetic control animals at the time of each experiment (Table 2 and Fig. 1). Plasma insulin levels were lower in alloxan-diabetic mice than in control mice 1 week after alloxan administration, with no progressive reduction in insulin levels over the 12-week study period.

Insulin-Induced Hypoglycemia

Plasma glucose and glucagon levels immediately before and for 1 hour after IP insulin administration in control and phloridzin-treated alloxan-diabetic animals at 1, 6, or 12 weeks after diabetes induction are illustrated in Fig 1. Plasma glucose levels were reduced similarly in response to insulin administration in control and alloxan-diabetic animals with a nadir of approximately 1 mmol/L at 30 minutes after insulin administration. In both control and alloxan-diabetic animals, a counterregulatory glucagon response was induced with a maximal increase observed at either 15 or 30 minutes after insulin administration. The hypoglycemia-induced increase in glucagon levels was not significantly different between control and diabetic animals after 1 week of diabetes. However, the increase in glucagon levels was smaller in the diabetic animals at 6 or 12 weeks after diabetes induction than in the controls. This is illustrated in Fig 2, which shows the AUC in the 2 groups at 1, 6, or 12 weeks after alloxan administration. The AUC in the diabetic animals was not significantly reduced at 1 week after alloxan administration, but was reduced by 60% after 6 weeks ($P = .021$) and by 70% after 12 weeks ($P = .008$). Thus, the A-cell response to insulin-induced hypoglycemia becomes impaired over the course of 12 weeks of alloxan-induced diabetes in mice.

Arginine

To determine whether there is a generalized impairment of A-cell function in alloxan-induced diabetes or whether there is an inability of the A cells to elicit a full glucagon response due to the elevated baseline glucagon levels, we also examined the glucagon responses to the amino acid, L-arginine. The glucagon responses to arginine at 1, 6, and 12 weeks after diabetes

Table 1. Plasma Glucose and Insulin Levels After an Overnight Fast and After an Additional 4-Hour Treatment of Control Animals With Vehicle or of Alloxan (60 mg/kg IV)-Diabetic Animals With Phloridzin

	1 Week		6 Weeks		12 Weeks	
	Controls	Alloxan-Diabetes	Controls	Alloxan-Diabetes	Controls	Alloxan-Diabetes
No. of animals	44	44	44	42	38	36
Fasting glucose (mmol/L)	4.8 ± 0.3	15.7 ± 1.3*	3.9 ± 0.4	12.6 ± 1.4	4.0 ± 0.3	14.6 ± 1.7*
Fasting insulin (pmol/L)	152 ± 32	36 ± 5*	146 ± 7	32 ± 3*	114 ± 11	38 ± 6*
Post vehicle (controls) or phloridzin (diabetics) glucose (mmol/L)	3.5 ± 0.2	3.6 ± 0.2 (NS)	3.5 ± 0.3	3.3 ± 0.4 (NS)	3.8 ± 0.3	3.7 ± 0.4 (NS)
Post vehicle (controls) or phloridzin (diabetics) glucagon (pg/mL)	51 ± 3	245 ± 30*	52 ± 5	586 ± 93*	53 ± 4	696 ± 101*

NOTE. Means ± SEM are shown.

Abbreviation: NS, not significant.

*Probability level of random difference between groups, $P < .001$.

induction in the mice are shown in Fig 3. There was no difference in the glucagon response to arginine between non-diabetic and alloxan diabetic mice at any of the time points studied. Therefore, there is no generalized impairment of A-cell function in alloxan-induced diabetes. Arginine increased plasma insulin levels in controls but not in alloxan-diabetic mice (Table 2).

2-DG

Since the impairment of the glucagon response to insulin-induced hypoglycemia in diabetes may be due to supersensitivity of the A cell to the suppressive effect of insulin, we examined whether the glucagon response to another glucopenic stimulus is also impaired. We thus administered the glucose analog, 2-DG, to the mice, since the stimulation of glucagon secretion by 2-DG is achieved in the alloxan-diabetic animals without any concomitant increase in circulating insulin. Figure 4 shows the 2-DG–induced glucagon response at 1, 6, and 12 weeks after alloxan or saline. At 1 week, the glucagon response to 2-DG did not differ between the diabetic and control animals, whereas after 6 or 12 weeks, the 2-DG–induced glucagon response was impaired by approximately 60% in the diabetic

animals ($P = .026$ and $P = .022$, respectively). 2-DG increased plasma insulin levels in controls, but not in alloxan-diabetic mice (Table 2). Therefore, the glucagon response to 2-DG was impaired at 6 to 12 weeks after induction of diabetes by alloxan, like that to insulin-induced hypoglycemia.

Carbachol

Both insulin-induced hypoglycemia and 2-DG–induced neuroglucopenia are known to activate the parasympathetic nerves innervating the pancreas, and to stimulate glucagon secretion by an atropine-sensitive mechanism.[6,29] To examine whether the impairment of 2-DG—induced glucagon secretion in alloxan-diabetic mice might be due to an impairment of the A cells response to cholinergic activation, we administered the cholinergic agonist, carbachol, at 1, 6, and 12 weeks after diabetes induction. As is seen in Fig 5, the glucagon response to carbachol was larger in alloxan-diabetic animals when compared with the controls after 1, 6, or 12 weeks after diabetes ($P < .05$ or less). Carbachol increased plasma insulin levels in controls but not in alloxan-diabetic mice (Table 2). Therefore, the responsiveness of the A cells to cholinergic activation is not reduced in alloxan-induced diabetes.

Table 2. Plasma Glucose Levels After an Overnight Fast Before and After IV Administration of 2-DG (0.5 g/kg), Arginine (0.25 g/kg), or Carbachol (0.53 μmol/kg), and the Insulin Response to 2-DG, Arginine or Carbachol

	1 Week		6 Weeks		12 Weeks	
	Controls	Alloxan-Diabetes	Controls	Alloxan-Diabetes	Controls	Alloxan-Diabetes
Glucose (mmol/L)						
Before 2-DG	3.3 ± 0.2	4.2 ± 0.3	3.6 ± 1.0	3.5 ± 1.0	4.1 ± 0.4	3.5 ± 0.6
Before arginine	4.1 ± 0.2	4.0 ± 0.8	3.5 ± 0.1	2.5 ± 0.3	4.1 ± 0.3	3.7 ± 0.8
After arginine	7.6 ± 0.4	6.9 ± 1.1	6.9 ± 0.7	2.7 ± 0.4	5.4 ± 0.4	4.2 ± 0.9
Before carbachol	4.1 ± 0.3	3.3 ± 0.3	4.1 ± 0.4	3.6 ± 0.7	4.5 ± 0.2	4.6 ± 1.5
After carbachol	7.6 ± 0.6	5.5 ± 0.7	6.8 ± 0.6	4.6 ± 1.2	5.5 ± 0.5	4.6 ± 1.2
Insulin response (pmol/L)						
2-DG	277 ± 96	45 ± 17	169 ± 74	19 ± 15	371 ± 60	29 ± 23
Arginine	338 ± 85	83 ± 32	258 ± 76	22 ± 8	584 ± 154	99 ± 81
Carbachol	330 ± 121	133 ± 90	396 ± 120	27 ± 10	318 ± 130	27 ± 17

NOTE. Experiments were performed after 4-hour treatment of control animals with vehicle and of alloxan (60 mg/kg IV)-diabetic animals with phloridzin to match glucose levels. Glucose levels after 2-DG are not interpretable because 2-DG partially crossreacts with glucose oxidase in the glucose assay. Means ± SEM are shown. There were 8 to 12 animals in each group.
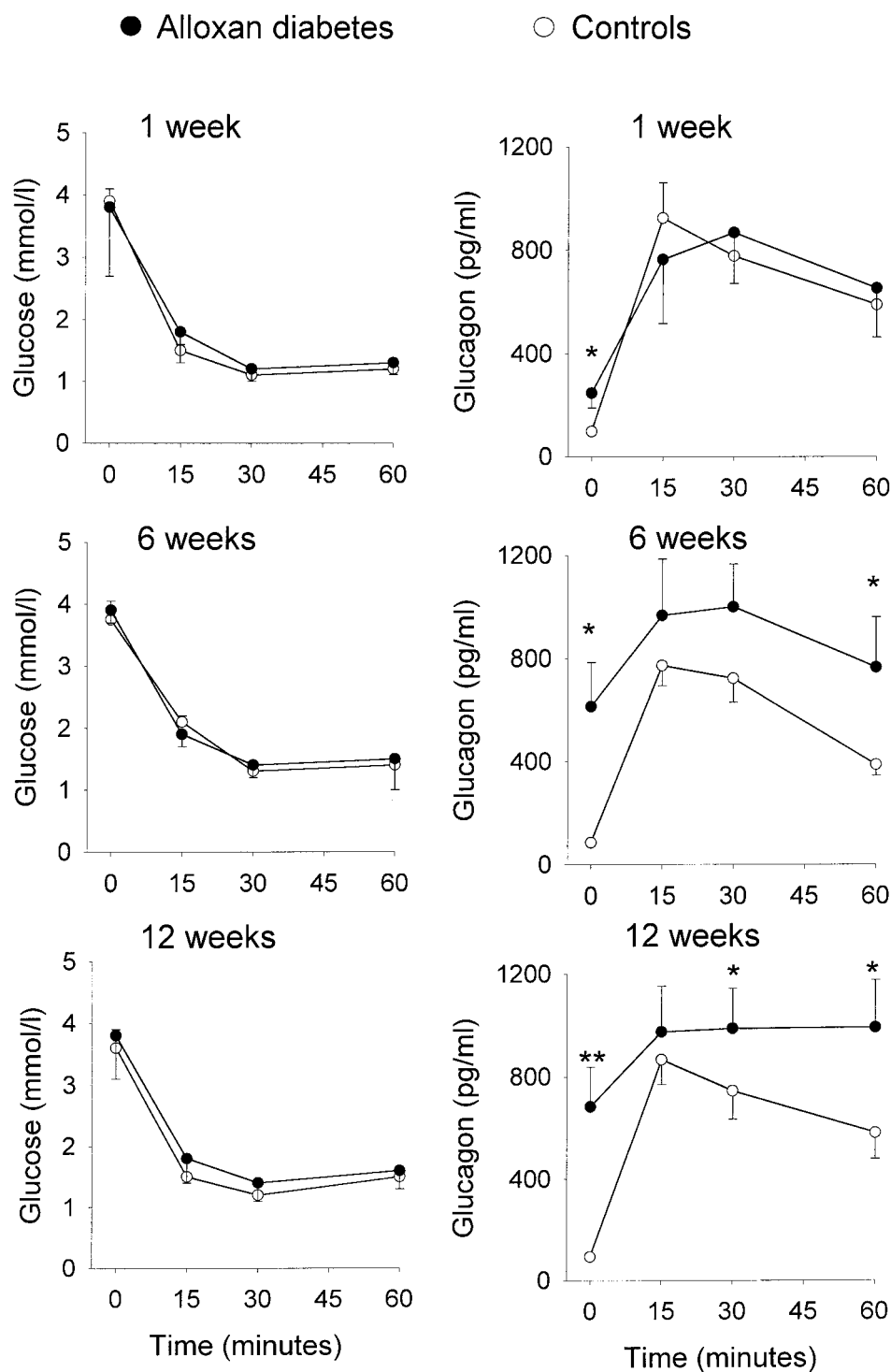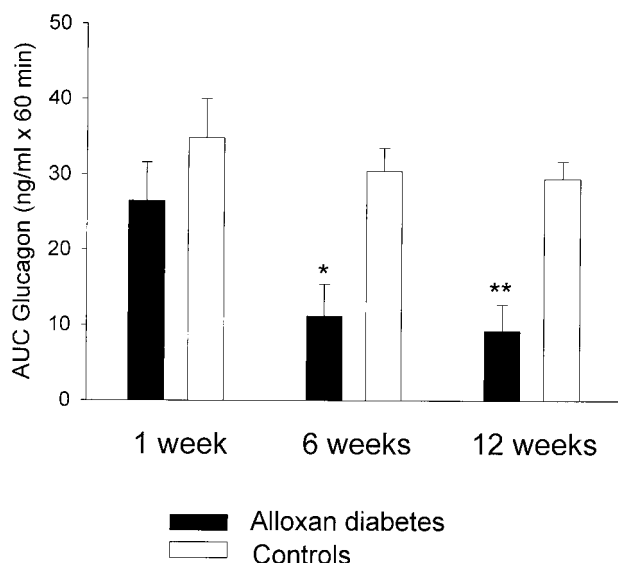
Fig 1. Plasma levels of glucose and glucagon before and after IP administration of insulin (2 U/kg) to overnight fasted control and alloxan-diabetic mice at 1, 6, or 12 weeks after administration of alloxan (60 mg/kg). Alloxan-diabetic animals had been treated with phloridzin 4 hours before the experiments, to match glucose levels at the time of insulin administration. Means ± SEM are shown. There were 10-12 animals in each group. Asterisks indicate the probability level of random difference between the groups at the indicated time periods. *$P < .05$; **$P < .01$.

DISCUSSION

In this study we used the alloxan diabetic mouse to examine the time-course of the impairment of the glucagon response to insulin-induced hypoglycemia after the induction of diabetes. In addition, glucagon responses to other stimuli were examined to gain insight into the mechanism(s) responsible for this im-

pairment. Since these glucagon responses are influenced by the baseline glucose levels present before the acute stiumlus,[18,30] it is important to match the glucose levels when comparing glucagon response between diabetic and nondiabetic animals. In this study, glucose concentrations were matched by administering phloridzin to the alloxan-diabetic mice. After acutely

Fig 2.   The 60-minute area under the glucagon curve (AUC$_{glucagon}$) after IV insulin (2 U/kg) in overnight fasted control and alloxan-diabetic mice at 1, 6, or 12 weeks after administration of alloxan (60 mg/kg). Alloxan-diabetic animals had been treated with phloridzin before the experiments, to match glucose levels at the time of insulin administration. Means ± SEM are shown. There were 10 animals in each group. Asterisks indicate the probability level of random difference between the groups. *P < .05, **P < .01.

normalizing the baseline glucose levels with phloridzin injections, insulin produced a marked degree of hypoglycemia (∼1 mmol/L), which was equivalent in control and alloxan diabetic animals. The hypoglycemia in turn induced an increase in circulating glucagon concentrations in both the diabetic and the nondiabetic animals, which was maximal at 15 to 30 minutes after insulin administration. To compare the glucagon responses between different groups at different times, we calculated the net increase in glucagon levels by subtracting the basal levels from the stimulated levels in each individual mouse. The conclusions are therefore based on the assumption that the increment over baseline concentrations is the appropriate index of the glucagon response to hypoglycemia and an important determinant of counterregulatory response, even when basal glucagon levels are elevated. However, since our study was focused on glucagon secretion, we did not follow the entire glucose counterregulatory response. Therefore, this study does not allow conclusions about the physiological action of the glucagon response in diabetic versus control mice. The study does show that while the glucagon response to insulin-induced hypoglycemia was not different between the 2 groups after 1 week of diabetes, there was an approximately 60% reduction of this glucagon response after either 6 or 12 weeks of diabetes. This result agrees with a previous longitudinal study in streptozotocin-diabetic rats showing a normal glucagon response to hypoglycemia after 1 week of diabetes, but a substantial impairment of the glucagon response after 6 weeks of diabetes.[12] Similarly, in cross-sectional studies, impaired glucagon responses to insulin-induced hypoglycemia have also been reported in humans,[3-8] dogs,[31] and rats[9,13,14,32] after dia-

betes of various durations, although the development of the impairment appears to occur more rapidly in rodents than in humans.

Several mechanisms have been proposed to account for the impairment of the glucagon response to insulin-induced hypoglycemia in type 1 diabetes, including (1) a generalized impairment of A-cell function, (2) a supersensitivity of the A cell to the suppressive effect of exogenous insulin, (3) an impaired A-cell responsiveness to autonomic stimulation, (4) an impairment of A-cell responsiveness to glucopenic stimulation and (5) an impaired activation of the autonomic inputs to the α cell during glucopenia.[5,15-18]

To test for a generalized impairment of A-cell function, we administered the amino acid, L-arginine. We found that the glucagon response to arginine in nondiabetic control animals was not different than that found in alloxan-diabetic mice, regardless of the duration of diabetes. Therefore, in this study alloxan-induced diabetes does not appear to be associated with a generalized defect in A-cell responsiveness. This finding is similar to that found in human diabetes mellitus of long duration: type 1 diabetic patients have a severe impairment of their glucagon response to insulin-induced hypoglycemia yet retain a normal glucagon response to arginine.[5] Thus, alloxan-diabetic mice model a major feature of glucagon secretion seen in human type 1 diabetes.

Another form of generalized impairment could possibly be caused by the elevated baseline glucagon levels seen after phloridzin treatment of alloxan-diabetic mice. This basal hyperglucagonemia may be caused by increased number of glucagon cells, which has been demonstrated in alloxan-diabetic mice,[33] insulin deficiency, or the reduction in glucose induced by phloridzin. High glucagon levels have also been reported in streptozotocin-diabetic rats.[34] Thus it is possible that the high baseline levels, particularly those seen after 12 weeks of diabetes, might have limited the magnitude of the calculated
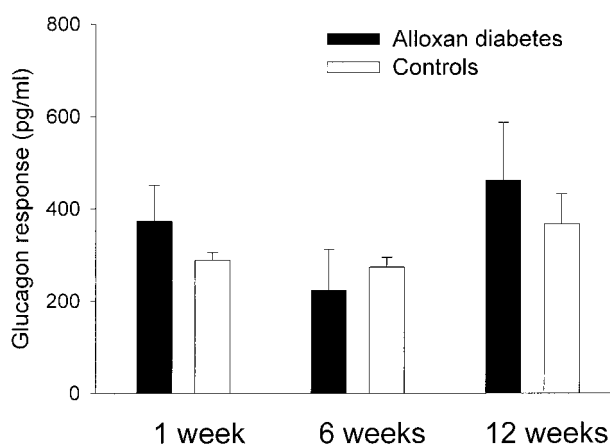
Fig 3.   Increase in plasma glucagon 2 minutes after IV administration of arginine (0.25 g/kg) in overnight fasted control and alloxan-diabetic mice at 1, 6, and 12 weeks after administration of alloxan (60 mg/kg). Alloxan-diabetic animals had been treated with phloridzin before the experiments, to match the glucose levels at the time of arginine administration. Means ± SEM are shown. There were 8-10 animals in each group.
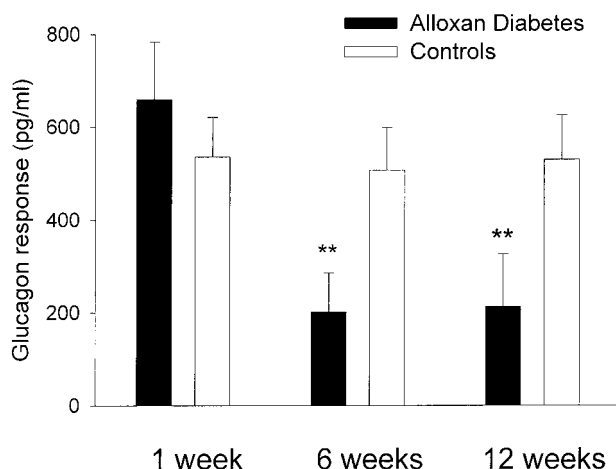
Fig 4. Increase in plasma glucagon 10 minutes after IV administration of 2-DG (0.5 g/kg) in overnight fasted control and alloxan-diabetic mice at 1, 6, and 12 weeks after administration of alloxan (60 mg/kg). Alloxan-diabetic animals had been treated with phloridzin before the experiments, to match glucose levels at the time of 2-DG administration. There were 10-12 animals in each group. Asterisks indicate the probability level of random difference between the groups. **$P < .01$.

glucagon response to acute stimulation, if the glucagon peak reached the maximal glucagon level obtainable. However, since the absolute peak glucagon levels were higher after arginine (or carbachol) than after insulin (or 2-DG) and since the baseline glucagon levels were similar before these acute stimuli, then the calculated glucagon response to insulin-induced hypoglycemia does not seem to be limited by the elevated baseline glucagon levels observed at 12 weeks of diabetes.

The second possible mechanism for the impairment of the glucagon response to insulin-induced hypoglycemia is the development of A-cell supersensitivity to the suppressive effects of exogenous insulin. We therefore examined the glucagon response to a glucopenic agent, 2-DG, which does not increase the circulating insulin level in alloxan-diabetic mice. Since we found that the glucagon response to 2-DG was also impaired at 6 and 12 weeks of diabetes we suggest that neither impairment is due to supersensitivity of the A cell to the suppressive action of exogenous insulin.

To test the possibility that the impaired glucagon response to insulin-induced hypoglycemia in the alloxan-diabetic mice might be caused by loss of sensitivity to direct stimulation by autonomic inputs, we administered the cholinergic agonist, carbachol. Carbachol is a potent agent stimulating glucagon secretion in mice through activation of muscarinic receptors: carbachol-stimulated glucagon secretion in mice is prevented by muscarinic antagonism.[24,25,26,35] Surprisingly, we found that carbachol-stimulated glucagon secretion was augmented in this particular species and duration of diabetes, suggesting supersensitivity to, rather than impairment of, direct cholinergic stimulation of the A cell in these animals. These data argue against an impaired responsiveness of the A cell to autonomic stimulation as a cause of the impaired glucagon response to insulin-induced hypoglycemia.

One possible mechanism that cannot be ruled out by the present studies is an impairment of the A cell's ability to respond to direct glucopenic stimulation. Such a mechanism is consistent with our finding of an impaired glucagon response to the 2 glucopenic stimuli, insulin-induced hypoglycemia and 2-DG, at 6 and 12 weeks of diabetes, as well as unimpaired glucagon responses to the nonglucopenic stimuli, arginine and carbachol. However, our previous studies have shown that ganglionic blockade nearly eliminated the glucagon response to insulin-induced hypoglycemia in normal mice,[35] suggesting that the direct glucopenic stimulation of the A cell produces only a minor increase of glucagon secretion. It is therefore unlikely that the impairment of the ability of the A cell to respond to direct glucopenic stimulation is responsible for the marked impairment of the glucagon response to insulin-induced hypoglycemia observed in diabetes.

A defective glucagon response to insulin-induced hypoglycemia in diabetes might also be explained by disturbed islet cell-cell interactions.[15] Thus, if inhibition of endogenous insulin secretion after administration of exogenous insulin is a stimulus for glucagon secretion, then alloxan-induced destruction of the islet B cells could impair the glucagon response to insulin-induced hypoglycemia. However, a previous study in humans has shown that prior marked suppression of endogenous insulin secretion does not potentiate the glucagon response to hypoglycemia,[36] which argues against a major role for inhibition of endogenous insulin secretion.

Further, since 2-DG stimulates insulin secretion in nondiabetic mice, suppression of endogenous insulin cannot play a significant role in the glucagon response to this glucopenic stimulus; therefore defects in B-/A-cell interactions in the alloxan-diabetic mice cannot explain its impairment. Another potential mechanism for the reduction of the glucagon response
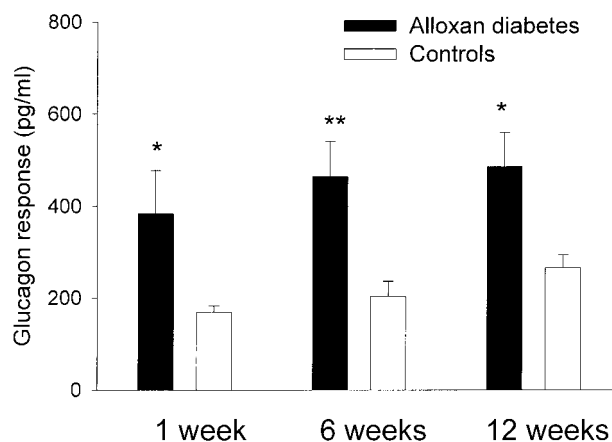
Fig 5. Increase in plasma glucagon 2 minutes after IV administration of the cholinergic agonist, carbachol (0.53 μmol/kg) in overnight fasted control and alloxan-diabetic mice at 1, 6, and 12 weeks after administration of alloxan (60 mg/kg). Alloxan-diabetic animals had been treated with phloridzin before the experiments, to match the glucose levels at the time of carbachol administration. Means ± SEM are shown. There were 8-10 animals in each group. Asterisks indicate the probability level of random difference between the groups and between the different time period within the groups; *$P < .05$, **$P < .01$.

to neuroglucopenic stimuli is an impairment in the activation of the autonomic inputs to the A cell. Indeed, in nondiabetic mice, activation of the autonomic inputs to the A cell has been demonstrated to be the major mediator of the glucagon response to both insulin-induced hypoglycemia and 2-DG–induced glucopenia since blockade of the ganglionic transmission involved in these autonomic pathways abolishes 90% of these responses.[35] Thus it remains possible that 6 to 12 weeks of diabetes can impair the autonomic activation normally elicited by either insulin-induced hypoglycemia or 2-DG resulting in the impaired glucagon response to each. Support for this hypothesis is provided by earlier studies in streptozotocin treated rats which demonstrated a parallel reduction in the autonomic activation and the glucagon response to insulin-induced hypoglycemia with increasing duration of diabetes.[12,13] Measurements of plasma epinephrine, as an index of the sympathoadrenal response, and plasma pancreatic polypeptide, as an index of the pancreatic parasympathetic response,[29] in the present study would have provided direct evidence bearing on this

potential autonomic mechanism. Unfortunately, the limited blood volume of these mice precluded such measurements. The only evidence in the present study for an autonomic impairment is very indirect: the glucagon response to carbachol is exaggerated in diabetic. This impairment could be secondary to islet parasympathetic denervation. Thus, although the known autonomic mediation of the glucagon response to insulin-induced hypoglycemia and 2-DG in nondiabetic mice coupled with the parallel reduction in the glucagon response to these same stimuli with increasing duration of diabetes in alloxan treated mice suggests that an autonomic impairment might mediate the impaired glucagon response to insulin-induced hypoglycemia; further studies are needed to definitely implicate this as the dominant mechanism response for the loss of the A-cell response.

ACKNOWLEDGMENT

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