

# Correction of hyperglycemia with phloridzin restores the glucagon response to glucose in insulin-deficient dogs: Implications for human diabetes

(alpha cells/glucose transporters/islet cell function/diabetic remission)

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**ABSTRACT** In insulin-deprived alloxan-induced diabetic dogs with severe hyperglycemia and marked hyperglucagonemia, glucagon was not suppressed by intravenous infusion of glucose at a progressively increasing rate up to 24 mg/kg of body weight per min. However, when the hyperglycemia was corrected by phloridzin, a blocker of renal tubular glucose reabsorption, the hyperglucagonemia was readily suppressed by as little as 2 mg of glucose per kg/min. Direct perfusion of phloridzin into the isolated pancreas of nondiabetic dogs had no effect on the *in vitro* glucagon response to increments in glucose. However, in pancreata isolated from dogs whose glucose levels had been lowered by phloridzin pretreatment, *in vitro* glucagon suppression in response to glucose increments was more than twice that of controls. This enhancing effect of phloridzin treatment was completely abolished by giving an intravenous infusion of glucose for the 5 hr prior to surgery for isolation of the pancreas. It is concluded that (i) alpha cells have a glucose-sensing system that is independent of insulin and beta cells, and (ii) this system is reversibly attenuated by hyperglycemia. Thus, hyperglycemia, a metabolic consequence of islet cell dysfunction, may be a self-exacerbating inducer of further islet cell dysfunction, a possibility with implications for human diabetes.

Whereas normally plasma glucagon levels are suppressed by hyperglycemia (1), the hyperglucagonemia that characterizes the diabetic state (1, 2) is dramatically suppressed by insulin (3) but not by glucose (2). This has suggested that pancreatic alpha cells lack their own glucose-sensing system and that in normals the suppressive effect of hyperglycemia upon glucagon secretion is mediated through increased secretion of insulin (4), a potent inhibitor of glucagon secretion (5). To examine this issue further, we studied the response of glucagon to glucose in dogs with severe alloxan-induced insulin deficiency both in the presence and in the absence of hyperglycemia. The results reveal the existence of a glucose-sensing system for alpha cells that functions in the absence of beta cells and of insulin—provided chronic hyperglycemia is not present. The latter proviso implies that chronic hyperglycemia, a consequence of impaired islet cell function, attenuates the alpha cell response to glucose and, thus, contributes to its own exacerbation.

## METHODS AND MATERIALS

Severe insulin-requiring diabetes mellitus was induced in six normal dogs by a single intravenous injection of 70–75 mg/kg of body weight of alloxan. Experiments were begun a minimum of 3 months thereafter. The daily insulin dose of 24

to 36 units of Isophane Insulin (Eli Lilly) was discontinued 88 hr before an experiment so as to produce a profound insulin deficiency. With the dogs in a conscious state, we infused glucose at 2, 4, 8, 12, 16, and 24 mg/kg per min for 20 min at each rate. On a second occasion the same dogs were studied after hyperglycemia had been corrected without administering insulin. This was accomplished by subcutaneously injecting 2 g of phloridzin (Sigma) as a 40% solution in propylene glycol on 2 consecutive days beginning 48 hr after discontinuation of insulin therapy. Experiments were begun 16 hr after the second phloridzin injection in both the diabetic dogs and in another group of nondiabetic control dogs that received phloridzin.

In other experiments perfusion of the isolated canine pancreas was carried out by the method of Iversen and Miles (6) as recently modified (7).

Glucagon was measured by the method of Harris *et al.* (8). Free insulin was measured by a modification (9) of the method of Yalow and Berson (10) following Sep-Pak extraction (11). Somatostatin-like immunoreactivity was measured by the method of Harris *et al.* (12).

## RESULTS

**In Vivo Studies.** Eighty-eight hours after the discontinuation of insulin treatment in alloxan-induced diabetic dogs, fasting plasma glucose levels averaged  $318 \pm 20$  mg/dl, 24-hr urinary glucose excretion averaged  $179 \pm 66$  g, and plasma glucagon levels averaged  $427 \pm 39$  pg/ml. Plasma-free insulin was undetectable. The intravenous infusion of glucose at a progressively increasing rate did not reduce glucagon levels significantly until the rate reached 24 mg/kg per min, at which time the mean plasma glucose level exceeded 600 mg/dl, or  $95 \pm 7\%$  above the fasting glucose level (Fig. 1A).

This unresponsiveness to glucose of the pancreatic alpha cells [gastric alpha cells do not respond to glucose (13, 14)] could have been the consequence of the hyperglycemia alone, the deficiency of insulin, or both. To assess the role of hyperglycemia alone, the same six dogs were studied in a normoglycemic but nevertheless insulin-deficient state by blocking renal glucose reabsorption with phloridzin, which binds to the sodium-dependent active glucose transporter in the brush border of renal tubular cells (15). Glycosuria increased to  $391 \pm 63$  g/24 hr, and the mean fasting glucose concentration declined from  $302 \pm 29$  mg/dl just before phloridzin treatment to  $65 \pm 7$  mg/dl on the day of the experiments. Plasma glucagon averaged  $970 \pm 186$  pg/ml, significantly greater than during hyperglycemia ( $P < 0.05$ ). Free insulin was undetectable. There were no other observed effects of phloridzin. Despite the absence of circulating insulin, statistically significant ( $P < 0.05$ ) suppression of glucagon

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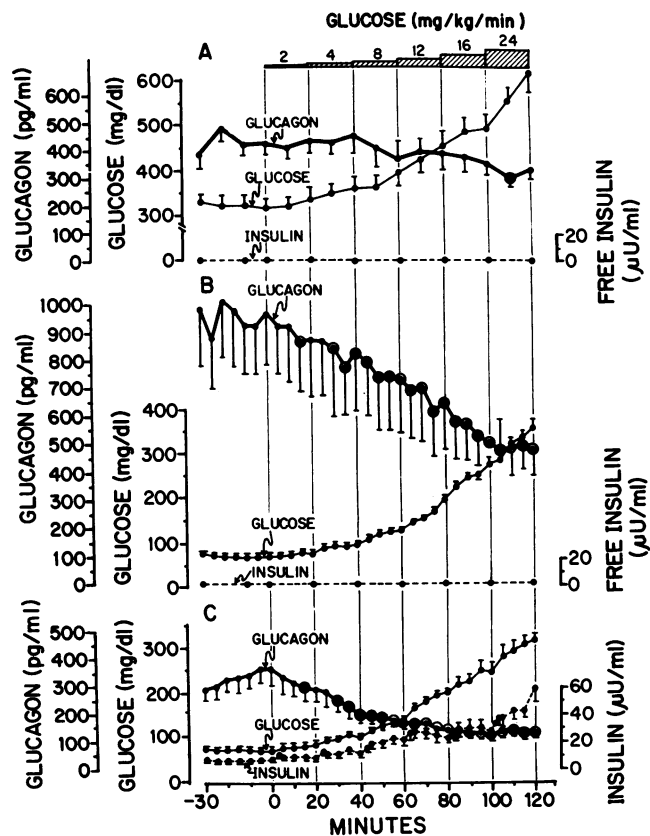


FIG. 1. The response of plasma glucagon levels (mean  $\pm$  SEM) to stepwise increments in the rate of intravenous infusion of glucose in six conscious insulin-deprived hyperglycemic alloxan-induced diabetic dogs (A), in the same six insulin-deprived alloxan-induced diabetic dogs made normoglycemic by phloridzin pretreatment (B), and in six nondiabetic dogs after phloridzin pretreatment (C). The experiments in the diabetic dogs were separated by an interval of at least 2 wk; in three dogs the hyperglycemic experiment was first and in three it was second. Circled points indicate a statistically significant difference from the mean baseline value at the 95% level by the paired *t* test.

gon first appeared during the infusion of glucose at the lowest rate, 2 mg/kg per min, which raised plasma glucose by only  $10 \pm 4$  mg/dl, or  $12 \pm 3\%$  of the fasting level (Fig. 1B). The cumulative glucagon response to the stepwise increase in glucose infusion was comparable to that of a group of six phloridzin-treated nondiabetic control dogs (Figs. 1C and 2). There was no significant change in plasma somatostatin-like immunoreactivity during glucose infusion in any of the experimental groups.

To determine if restoration of the glucagon response to glucose in insulin-deficient dogs was the consequence of a direct effect of phloridzin or of the reduction in plasma glucose levels that it induced, we perfused  $100 \mu\text{M}$  phloridzin into the isolated pancreata of six normal dogs. No evidence of enhancement by phloridzin of alpha cell sensitivity to an increase in perfusate glucose concentration was apparent (Table 1). However, pancreata isolated from normal dogs 48 hr after phloridzin treatment, which lowered their preoperative fasting glucose to  $66 \pm 12$  mg/dl as compared to  $98 \pm 7$  mg/dl in the controls, exhibited more than 2-fold enhancement of the glucagon response to an increase in perfusate glucose (Table 2). When glucose was infused in phloridzin-treated dogs at a rate of 19–21 mg/kg per min for 5 hr prior to resecting the pancreas for the perfusion experiment, the enhanced *in vitro* glucagon response to glucose increments was completely abolished (Table 2).

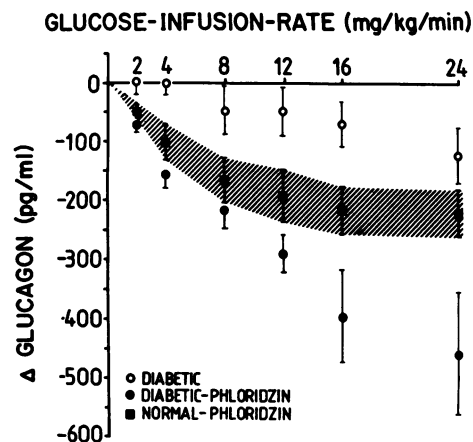


FIG. 2. The data from Fig. 1 replotted to show cumulative reduction in glucagon (mean  $\pm$  SEM) as a function of glucose infusion rate. The hatched area brackets the normal mean  $\pm$  SEM.

### DISCUSSION

The *in vivo* results indicate that suppression of glucagon by glucose, which is absent in uncontrolled diabetes, can be restored despite continuing insulin deficiency if hyperglycemia is abolished by phloridzin treatment. The *in vitro* studies provide no evidence that phloridzin has a direct sensitizing effect upon the alpha cell response to glucose when perfused into the isolated dog pancreas. However, the glucagon response to glucose increments is enhanced in pancreata from phloridzin-pretreated nondiabetic dogs, an effect that is abolished by an *in vivo* glucose infusion. These results imply that the enhancement is the consequence of the phloridzin-induced reduction in glucose levels prior to removal of the pancreas.

The study provides the first unequivocal evidence for the existence of a glucose sensing capability in alpha cells in the absence of insulin and beta cells. The existence of such a system had previously been doubted (4) because of the unresponsiveness of alpha cells to glucose during insulin deficiency and because insulin so rapidly and profoundly suppresses diabetic hyperglucagonemia (2). However, correction of hyperglycemia without insulin<sup>†</sup> causes an increase in hyperglucagonemia above that observed in the hyperglycemic insulin-deficient dogs. Perhaps this additional hyperglucagonemia, which is so sensitive to infused glucose, had already been suppressed in the non-phloridzin-treated diabetic dogs by their hyperglycemia. If so, this would suggest that

Table 1. Glucagon response (mean  $\pm$  SEM) to glucose increments in the isolated perfused canine pancreas (6): Effects of *in vitro* phloridzin perfusion

Glucose increase,* mg/dl		Glucagon decrease, pg/ml	
		Control (n = 7)	Phloridzin perfusion <sup>†</sup> (n = 6)
From	To		
25	50	NS	NS
50	100	$138 \pm 36$	$180 \pm 34$
100	150	$32 \pm 13$	$31 \pm 8$
150	300	$32 \pm 9$	$28 \pm 6$

NS, not significant.

\*The glucose concentration was raised at 15-min intervals.

<sup>†</sup>With  $100 \mu\text{M}$  phloridzin.

<sup>†</sup>Similar results have been reported recently following correction of hyperglycemia with insulin (16).

Table 2. Glucagon response (mean  $\pm$  SEM) to glucose increments in the isolated perfused pancreas of normal control dogs, normal dogs treated with phloridzin, and phloridzin-treated dogs who received a 5-hr glucose infusion before removal of the pancreas

Glucose increase,* mg/dl		Glucagon decrease, pg/ml		
		Control (n = 6)	Phloridzin pretreatment (n = 5)	Phloridzin pretreatment + 5-hr glucose infusion (n = 5)
From	To			
50	75	52 $\pm$ 20	146 $\pm$ 47 <sup>†</sup>	35 $\pm$ 7
75	100	38 $\pm$ 12	131 $\pm$ 24 <sup>‡</sup>	31 $\pm$ 6
100	150	14 $\pm$ 5	42 $\pm$ 9 <sup>‡</sup>	22 $\pm$ 2
150	200	4 $\pm$ 2	26 $\pm$ 14	2 $\pm$ 4

\*The perfusate glucose concentration was raised at 15-min intervals.

<sup>†</sup>P < 0.05 as determined by Student *t* test for two groups.

<sup>‡</sup>P < 0.01.

diabetic hyperglucagonemia in the insulinopenic state is initiated and maintained by the insulin deficiency (17). Alternatively or additionally, the hyperglycemia may have down-regulated a glucose transporter, as suggested by studies in other tissues (18–20) or altered post-transporter events. Conversely, the restoration of sensitivity to glucose following reduction in glycemia may reflect either an increase in glucose transporters comparable to that observed in glucose-starved chicken embryo fibroblasts during glucose starvation (21) and/or a freeing of previously occupied glucose-transport units or post-transporter amplification.

Steady-state hyperglycemia blunts the response of beta cells to further glucose increments (22). This may explain the progressiveness of the metabolic deterioration in inadequately treated diabetic states. The improvement in the insulin response to glucose challenge reported in type II diabetics following correction of hyperglycemia, whether through dietary carbohydrate restriction (23), treatment with sulfonyleureas (24), or insulin treatment (25–27), could be attributed to reversal of hyperglycemia-induced desensitization of beta cells.

The notion that hyperglycemia is both a consequence and an inducer of islet cell dysfunction would provide a long-sought link (28–30) between antecedent peripheral target tissue resistance to insulin action at the post-receptor level (31) and the gradual decline in islet function that culminates in overt type II diabetes. The same mechanism would also explain the severe metabolic deterioration that can occur in type I diabetes prior to complete destruction of beta cells and the high rate of transient remissions reported in such cases following early normalization of glycemia by means of the artificial pancreas (Biostator) (32).

The relationships between the blood glucose level and the diabetic state were first scrutinized by Soskin and Levine in 1937 (33). Diabetes was subsequently induced by prolonged intraperitoneal glucose injection in cats by Dohan and Lukens (34), who reported reversal of other forms of pituitary diabetes by elimination of hyperglycemia (35, 36). The present studies may help explain those early observations at the hormonal level.

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