

# Reversal of D- and A-Cell Insensitivity to Glucose in Alloxan-diabetic Dogs by Treatment with the Artificial Beta Cell (Biostator)

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## SUMMARY

Insulin-deficient diabetes in man as well as in experimental diabetes is associated with islet cell insensitivity to glucose. The present study was designed to determine whether this abnormality could be counteracted either by increasing the intraislet insulin level or by normalizing the diabetic state by a glucose-controlled insulin infusion system (GCIS: Biostator, Life Science Instruments, Elkhart, Indiana). Using the isolated, perfused pancreas of dogs with moderate, untreated alloxan diabetes of 4 days duration, we found that 5 mM arginine (N = 4) and 5 mM calcium (N = 4) stimulated D- and A-cell secretion, whereas an increment in glucose from 1.3 to 11 mM (N = 4) had no effect on islet hormone secretion. In the pancreas from untreated alloxan-diabetic dogs, acute infusion of large amounts of insulin (25 mU/ml) in vitro simultaneously with an elevation of perfusate glucose from 1.3 to 11 mM failed to restore the glucose sensitivity. In contrast, treatment of alloxan-diabetic dogs (N = 3) by a GCIS for 24 h revived some responsiveness of the glucagon, insulin, and somatostatin to glucose (1.3–11 mM) of the subsequently perfused pancreas. It is concluded that the insensitivity to glucose of islet cells in insulin-deficient diabetes is not ascribed to an intraislet insulin deficiency per se but rather to an abnormal metabolic state secondary to insulin deficiency. The results also indicate that the glucose receptor dysfunction is not due to a direct lesion by the diabetogenic drug. *DIABETES* 1985; 34:260–66.

Under normal conditions the pancreatic D-, A-, and B-cells are extremely responsive to changes in the extracellular glucose concentration.<sup>1–3</sup> Thus, an increase stimulates somatostatin and insulin and inhibits glucagon secretion, whereas a fall suppresses somatostatin and insulin and enhances secretion of glucagon.<sup>1–3</sup> In streptozocin (STZ) diabetes, however, variations in the glucose level fail to change pancreatic D- and A-cell release.<sup>4–7</sup> This loss of glucose-mediated control of the pan-

creatic D- and A-cell function in insulin-deficient diabetes appears to be a selective one, inasmuch as somatostatin and glucagon retain at least some responsiveness to other substances.<sup>3–9</sup> The abnormal pancreatic D- and A-cell function may be caused either by an intraislet insulin deficiency per se or by metabolic aberrations secondary to insulin deficiency. In addition, acute administration of alloxan into the pancreas in vitro has appeared to induce immediate alterations in hormone release,<sup>10</sup> so it remains to be disproved that alloxan induces a direct, possibly permanent damage of the glucoreceptor.

The aims of the present study were to examine: (1) the effects of arginine, calcium, and glucose on the release of somatostatin, glucagon, and insulin from the isolated perfused pancreas of dogs with another type of insulin-deficient diabetes, alloxan diabetes; (2) the relationship between the intraislet insulin level and the D- and A-cell responses to glucose in pancreata from alloxan-diabetic dogs; and (3) to investigate whether 24-h blood glucose normalization by in vivo treatment of alloxan-diabetic dogs using the artificial pancreas (Biostator, Life Science Instruments, Elkhart, Indiana) might restore pancreatic D- and A-cell function subsequently studied in vitro.

## MATERIALS AND METHODS

Eleven mongrel dogs weighing 17–28 kg were used in the study. Diabetes was induced in 7 dogs with alloxan (50 mg/kg) given as an i.v. bolus injection. The diabetic dogs were given a normal diet and i.v. saline treatment (500–1000 ml/day) for 3 days. Then they were divided into two groups. The following 24 h one group (A) remained without antidiabetic treatment (N = 4) while the other group (B) was treated with the artificial pancreas (Biostator) (N = 3). The latter dogs were briefly anesthetized with nembutal during connection to the glucose-controlled insulin infusion system

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TABLE 1

Alloxan diabetes: in 7 dogs diabetes was induced with an alloxan injection (50 mg/kg) 4 days before removal of the pancreas

	Before alloxan (mean $\pm$ SEM)	72 H after alloxan (mean $\pm$ SEM)	2P
Blood glucose (mmol/L)	4.4 $\pm$ 0.1	14.1 $\pm$ 1.6	<0.001
Plasma glucagon (pg/ml)	83 $\pm$ 22	139 $\pm$ 23	<0.01
Serum insulin ( $\mu$ U/ml)	24 $\pm$ 3	11 $\pm$ 2	<0.01

(GCIIS) (Biostator) and were in light N<sub>2</sub>O analgesia for 24 h until the operation. All dogs were anesthetized with nembutal anesthesia. The Biostator was used according to the instruction from the manufacturer. During the 24 h a total amount between 53 and 87 IU of insulin was delivered by the device.

Blood samples were obtained from the animals after an overnight fast just before alloxan injection and 72 h later. Venous blood from a foreleg was collected into tubes containing 10,000 KIU/ml Trasylol (FBA Pharmaceutical, New York, New York) and 3 mg/ml EDTA (pH = 8) and kept on ice for up to 20 min. The plasma was separated by centrifugation and stored at  $-18^{\circ}\text{C}$  until the assay of glucagon

and insulin. Blood for glucose determination was collected in tubes with sodium fluoride and stored at  $-18^{\circ}\text{C}$  until analysis.

All dogs were subjected to the same surgical procedure. Immediately before operation, the insulin-treated dogs were disconnected from the artificial pancreas. The technique for isolation of the pancreas and the perfusion system have previously been described in detail.<sup>11</sup> In brief, the preparation consisted of the pancreas and the proximal 10 cm of the attached duodenum. A nonrecirculating Krebs-Ringer bicarbonate buffer containing 40 g/L dextran (mol wt 75,000), 2 g/L bovine albumin, and glutamate, fumarate, and pyruvate (each 5 mM) was pumped through the splenic and coeliac arteries. The total portal effluent was collected every minute. Unless otherwise indicated, glucose was not added to the medium.

The perfusate was oxygenated by a rotating roller screen in an atmosphere at 94.4% O<sub>2</sub> and 5.6% CO<sub>2</sub>. The perfusion fluid was kept at 37°C and a constant pH of 7.4, its perfusion pressure being 30–40 mm Hg and flow rate 20 ml/min.

**Experimental protocol.** Samples were taken every 1 min from the efflux. To prevent possible degradation of somatostatin, glucagon, and insulin in the effluent, EDTA 3 mg/ml (pH = 8) was added to the collecting tubes. The samples were immediately stored at  $-18^{\circ}\text{C}$  until analysis.

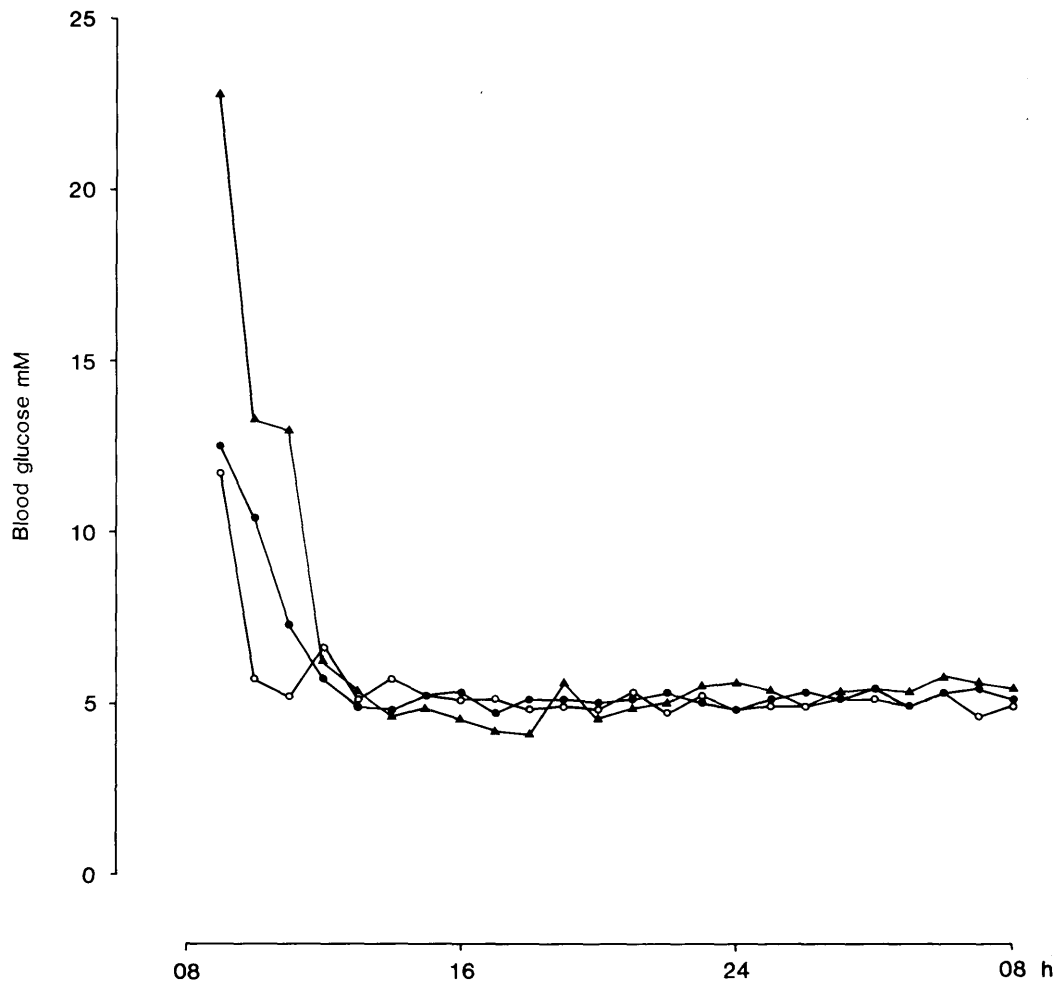
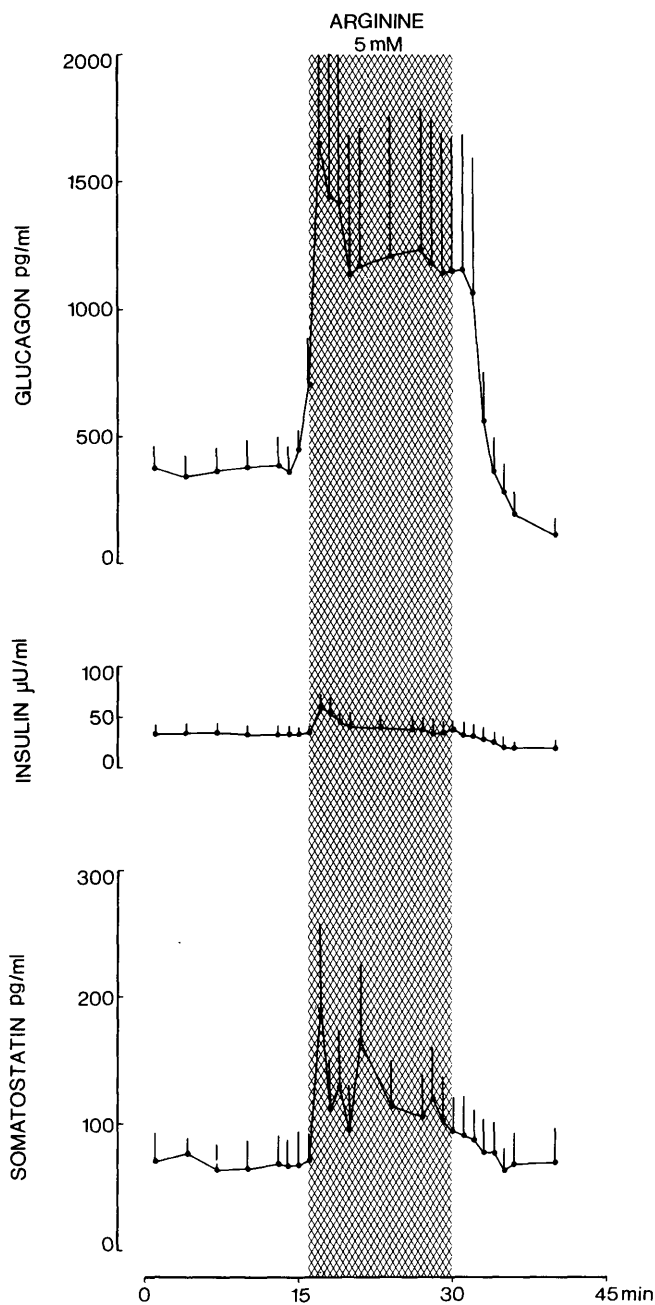


FIGURE 1. Blood glucose levels in 3 alloxan-diabetic dogs during 24-h treatment by a glucose-controlled insulin infusion system (GCIIS).



**FIGURE 2.** Effect of 5 mM arginine on glucagon, insulin, and somatostatin secretion from the isolated, perfused pancreas of untreated alloxan-diabetic dogs (N = 4). The glucose concentration was 11 mM. The data are shown as mean ± SEM.

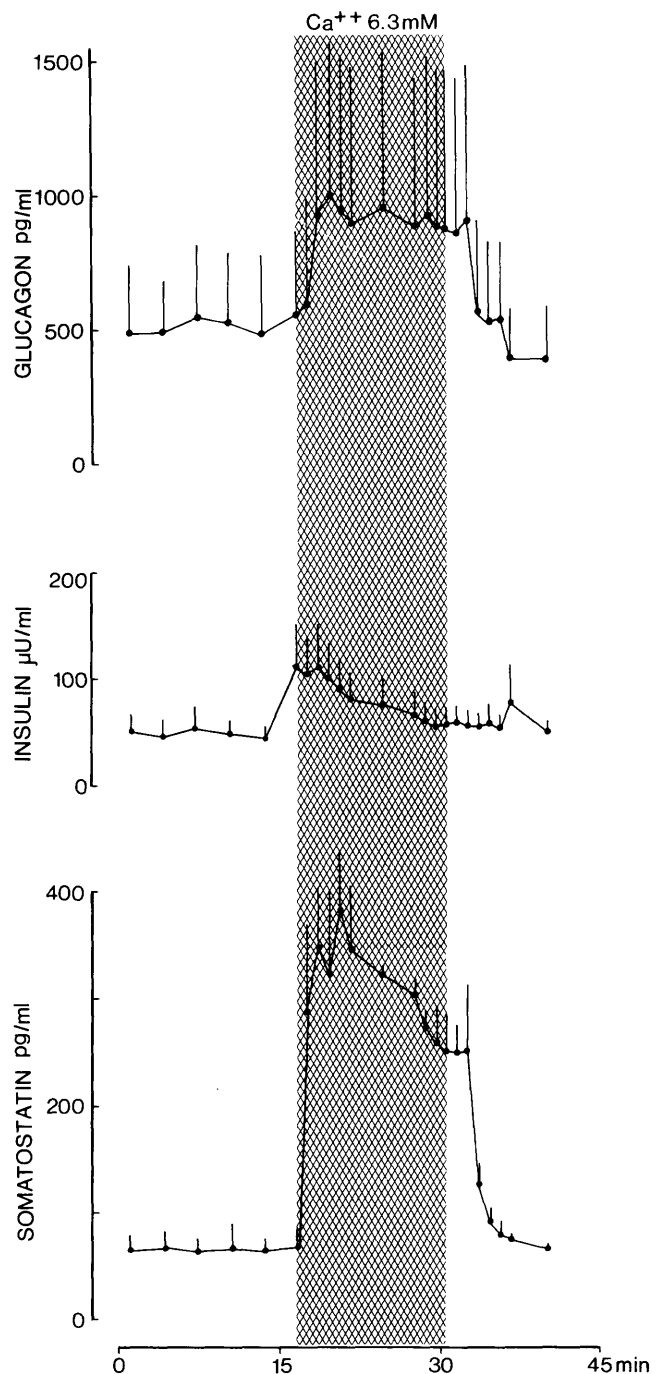
Each pancreas was perfused for an equilibration period of 20–30 min, then the test substances were infused for 15 min with 20–30-min recovery intervals. Each perfusion averaged 3 h.

**Analytic methods.** Somatostatin was measured by radioimmunoassay as previously described,<sup>12–14</sup> using the tyrosine-11 analogue of somatostatin iodinated with <sup>125</sup>I. The perfusion buffer was used as diluent for the standards. The detection limit was 2 pg/ml. Insulin and glucagon were measured by specific sensitive radioimmunoassays as described previously.<sup>15</sup> A pancreatic glucagon specific anti-serum (Lise Heding, Novo Research Institute, Copenhagen, Denmark) was used. Glucose was determined by a glucose-oxidase

method<sup>16</sup> and urine tested for ketones by Ketostix (Ames, Elkhart, Indiana).

**Calculations.** The percentage change in hormone secretion ( $\Delta\%$ ) was calculated from the mean of the 1-min hormone values during the entire carbohydrate infusion (B) and the mean of the last five 1-min values just before the addition of the carbohydrate (A) as

$$\Delta\% = \frac{B - A}{A} \times 100\%.$$



**FIGURE 3.** The effect of an increase in perfusate calcium from 1.3 to 6.3 mM upon islet hormone secretion from the isolated pancreas of untreated alloxan-diabetic dogs (N = 4). The glucose level was 11 mM. The data are shown as mean ± SEM.

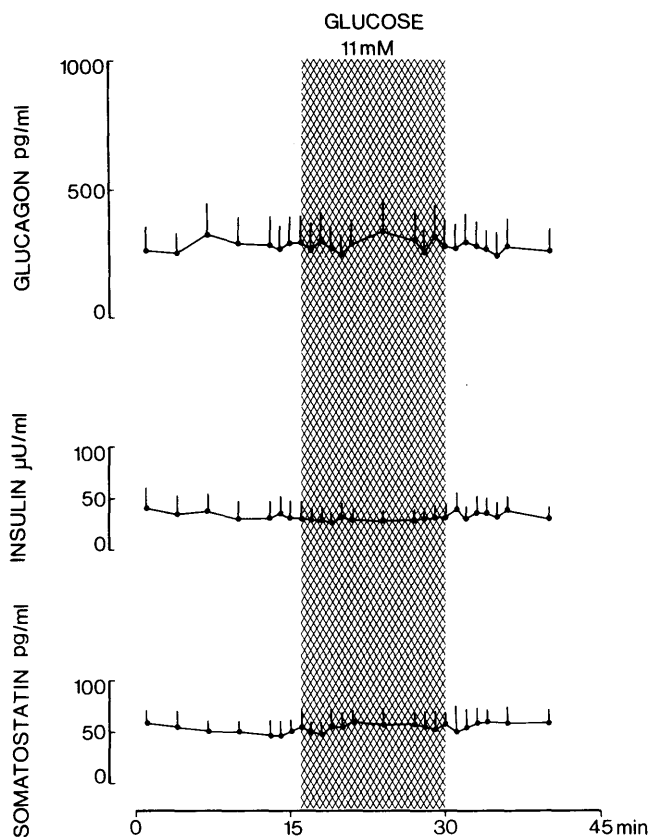


FIGURE 4. Lack of effect of elevation in perfusate glucose from 1.3 to 11 mM on glucagon, insulin, and somatostatin secretion from pancreas of untreated alloxan-diabetic dogs (N = 4; mean  $\pm$  SEM).

Data are given as mean  $\pm$  SEM. Statistical analyses were made by a two-tailed paired Student's *t*-test with a 5% limit of significance.

## RESULTS

### IN VIVO

Alloxan produced a moderate diabetic state with wasting and polyuria. As seen in Table 1 average blood glucose increased by a factor of three. Plasma glucagon increased also after alloxan treatment whereas the circulating insulin level was subdued. In three dogs treated for 24 h by the artificial pancreas, blood glucose was normalized for about 20 h (Figure 1). The amounts of insulin that had to be given were as large as 53–87 IU.

### IN VITRO

**Pancreatic islet cell function in untreated alloxan-diabetic dogs (N = 4).** Effects of arginine: The effects of 15 min perfusion of 5 mM arginine on somatostatin, glucagon, and insulin release from pancreas of untreated diabetic dogs were studied at 11 mM glucose (Figure 2). Arginine stimulated somatostatin (by  $82 \pm 9\%$ ,  $2P < 0.01$ ), glucagon (by  $290 \pm 66\%$ ,  $2P < 0.05$ ), and insulin secretion (by  $51 \pm 15\%$ ,  $2P < 0.05$ ).

Effects of calcium: The effect of increasing the extracellular calcium from 1.3 to 6.3 mM was studied in the presence of 11 mM glucose (Figure 3). Also, calcium elicited an aug-

mentation in the release of somatostatin (by  $457 \pm 99\%$ ,  $2P < 0.05$ ), glucagon (by  $93 \pm 22\%$ ,  $2P < 0.05$ ), and insulin (by  $34 \pm 5\%$ ,  $2P < 0.01$ ).

Effects of glucose: In contrast, as demonstrated in Figure 4, a shift in the extracellular glucose from 1.3 to 11 mM caused no change in somatostatin ( $3 \pm 4\%$ , NS), glucagon ( $2 \pm 2\%$ , NS), or insulin release ( $0 \pm 4\%$ , NS).

Effects of exogenous insulin: Figure 5 gives the islet hormone secretion in the effluent when perfusate glucose is increased from 1.3 to 11 mM. The simultaneous infusion of exogenous insulin (25 mU/ml) induced no change in somatostatin ( $1 \pm 3\%$ , NS) or glucagon output ( $8 \pm 3\%$ , NS).

**Pancreatic islet cell function in insulin-treated alloxan-diabetic dogs (N = 3).** Effects of glucose: Figure 6 shows the effect of glucose on somatostatin, glucagon, and insulin from pancreas of the dogs treated with the artificial pancreas. The alteration in perfusate glucose from 1.3 to 11 mM in these animals induced a suppression in glucagon (by  $15 \pm 2\%$ ,  $2P < 0.05$ ); an increase in somatostatin ( $60 \pm 5\%$ ,  $2P < 0.01$ ), and insulin ( $279 \pm 64\%$ ,  $2P < 0.05$ ).

The raw data from each dog appear in Table 2, which includes a comparison to results obtained in nondiabetic dogs.

Effects of exogenous insulin: When glucose was increased from 1.3 to 11 mM concomitantly with the addition of exogenous insulin (25 mU/ml), the output of glucagon was

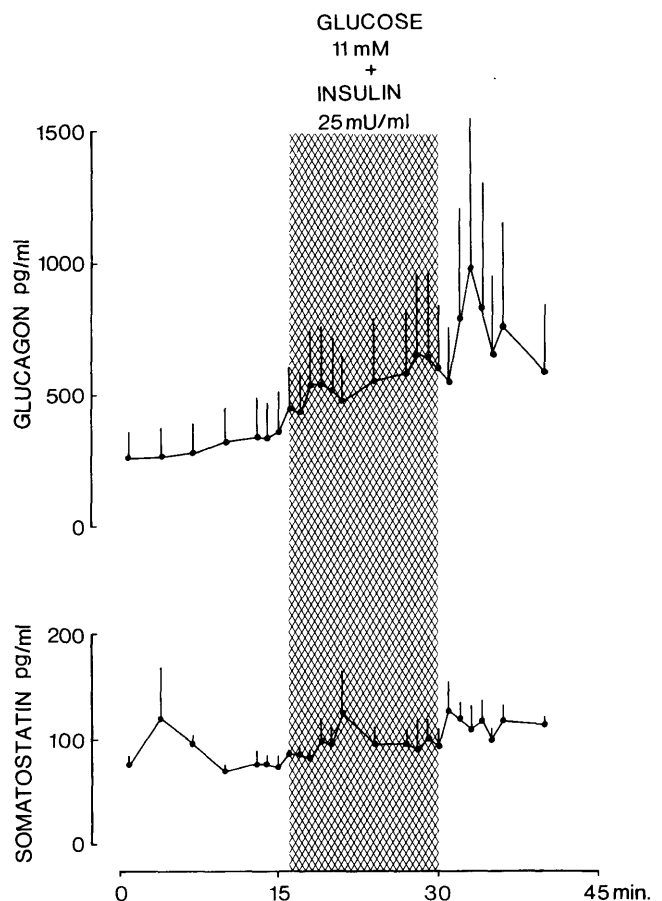
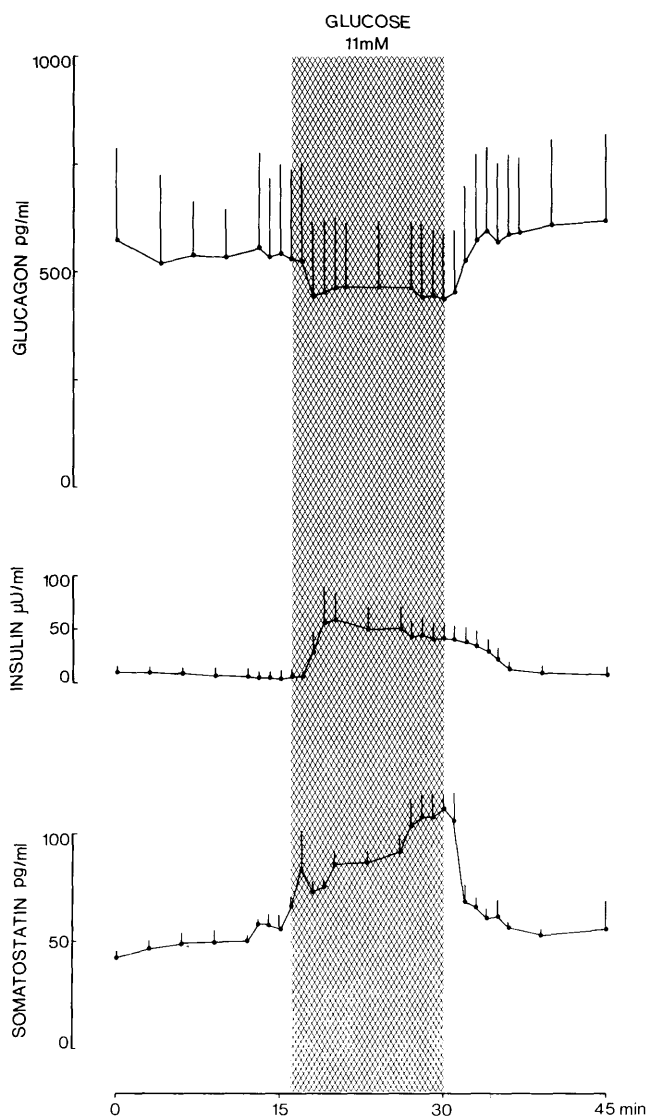


FIGURE 5. Effect on islet hormone secretion of the concomitant elevation of perfusate glucose (from 1.3 to 11 mM) and addition of exogenous insulin (25 mU/ml) in untreated alloxan-diabetic dogs (N = 4; mean  $\pm$  SEM).



**FIGURE 6.** Effect of an increase in perfusate glucose from 1.3 to 11 mM upon islet hormone secretion from pancreas of alloxan-diabetic dogs (N = 3) treated for 24 h by the artificial pancreas (mean  $\pm$  SEM).

inhibited (by  $21 \pm 2\%$ ,  $2P < 0.05$ ) and the output of somatostatin enhanced (by  $43 \pm 4\%$ ,  $2P < 0.01$ ) as seen in Figure 7, i.e., results identical to those obtained without simultaneous insulin infusion and different from those of untreated diabetic dogs.

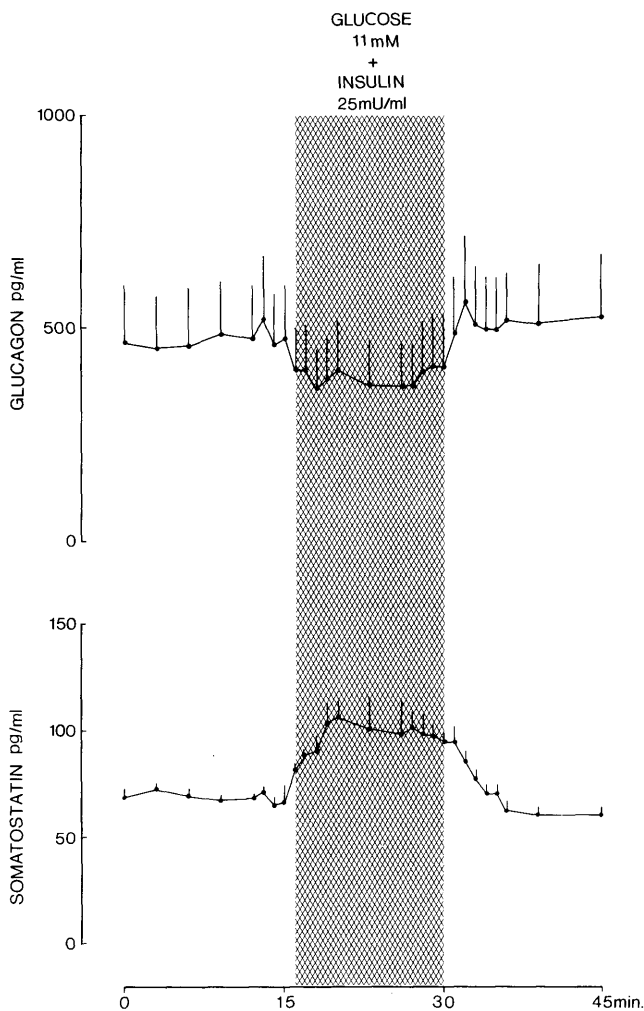
Average changes in islet hormone output to glucose: for comparison, the effect of a 15-min change in extracellular glucose from 1.3 to 11 mM was investigated in four normal pancreas perfusions. Figure 8 and Table 2 illustrate the average and individual changes in islet hormone output. Alterations in release of hormones from pancreas of untreated alloxan-diabetic (N = 4) and insulin-treated, alloxan-diabetic dogs (N = 3) were compared with those obtained in the normal pancreas. Data showing changes in absolute terms are depicted in the left panel and expressed in relative terms at the right panel of Figure 8. The D- ( $2P < 0.001$ ) and A-cell responses ( $P < 0.05$ ) after insulin treatment were greater than the (extinct) responses in the untreated group. The D-cell responses after insulin treatment did not signifi-

cantly differ from the normal responses. The percentage glucagon changes to glucose in the GCIIS-treated group, however, did not reach normal values. As expected, insulin responses to glucose in the GCIIS-treated group did not attain normal or near-normal responses.

**DISCUSSION**

The present results in untreated alloxan-diabetic dogs agree with previous results obtained in STZ-diabetic dogs.<sup>5,6</sup> Thus, the pancreatic islet cell responses to arginine and calcium in untreated alloxan diabetes were indistinguishable from the responses obtained in normal dogs,<sup>12</sup> whereas regulation by ambient glucose of somatostatin as well as glucagon was abolished. This indicates that selective insensitivity to glucose is present in alloxan diabetes as we have previously demonstrated in STZ diabetes.<sup>3,5,6</sup> Similar results have been obtained in insulin-deficient rats.<sup>1,7,17,18</sup>

The question arises whether the islet cell insensitivity to glucose is due to an intraislet insulin deficiency per se or the abnormal metabolic state secondary to insulin deficiency. To test the first possibility, we studied if the simul-



**FIGURE 7.** Effect of islet hormone secretion of the concomitant elevation of perfusate glucose (from 1.3 to 11 mM) and addition of 25 mU/ml exogenous insulin in alloxan-diabetic dogs treated for 24 h by the artificial pancreas (mean  $\pm$  SEM).

TABLE 2  
Effect of an increase in perfusate glucose from 1.3 to 11 mM upon pancreatic hormone secretion in normal, untreated, and GCIIS-treated alloxan-diabetic dogs

Group	Exp. no.	Exogenous insulin perfusion	Glucagon (pg/ml)			Insulin ( $\mu$ U/ml)			Somatostatin (pg/ml)		
			Before*	During†	$\Delta\%$	Before*	During†	$\Delta\%$	Before*	During†	$\Delta\%$
Control	1	Without	192 $\pm$ 18	75 $\pm$ 12	-61%	21 $\pm$ 3	281 $\pm$ 42	1238%	62 $\pm$ 4	120 $\pm$ 20	94%
	2		85 $\pm$ 5	21 $\pm$ 3	-75%	18 $\pm$ 2	358 $\pm$ 63	1889%	64 $\pm$ 5	107 $\pm$ 8	67%
	3		98 $\pm$ 7	22 $\pm$ 4	-78%	8 $\pm$ 4	181 $\pm$ 20	2163%	39 $\pm$ 7	89 $\pm$ 5	128%
	4		101 $\pm$ 14	31 $\pm$ 10	-69%	30 $\pm$ 2	921 $\pm$ 89	2970%	40 $\pm$ 3	97 $\pm$ 5	143%
	Mean $\pm$ SE			71 $\pm$ 4%, 2P < 0.001		2065 $\pm$ 359%, 2P < 0.05		108 $\pm$ 17%, 2P < 0.01			
Alloxan diabetes (untreated)	5	Without	61 $\pm$ 4	64 $\pm$ 7	4%	36 $\pm$ 3	34 $\pm$ 1	-6%	72 $\pm$ 2	83 $\pm$ 3	15%
	6		180 $\pm$ 5	173 $\pm$ 4	-4%	5 $\pm$ 0	5 $\pm$ 0	0%	29 $\pm$ 2	28 $\pm$ 2	-3%
	7		451 $\pm$ 30	483 $\pm$ 37	7%	67 $\pm$ 4	63 $\pm$ 12	-6%	38 $\pm$ 2	38 $\pm$ 1	0%
	8		408 $\pm$ 12	417 $\pm$ 11	2%	16 $\pm$ 1	18 $\pm$ 2	13%	69 $\pm$ 2	70 $\pm$ 2	1%
	Mean $\pm$ SE		2 $\pm$ 2%, NS		0 $\pm$ 4%, NS		3 $\pm$ 4%, NS				
	5	With	58 $\pm$ 3	63 $\pm$ 3	9%				72 $\pm$ 2	73 $\pm$ 2	1%
	6		176 $\pm$ 4	175 $\pm$ 5	-1%				46 $\pm$ 3	48 $\pm$ 2	4%
	7		615 $\pm$ 24	703 $\pm$ 128	14%				94 $\pm$ 8	101 $\pm$ 7	7%
8	547 $\pm$ 13		602 $\pm$ 11	10%				118 $\pm$ 3	107 $\pm$ 6	-9%	
Mean $\pm$ SE		8 $\pm$ 3%, NS					1 $\pm$ 3%, NS				
Alloxan diabetes (treated)	9	Without	213 $\pm$ 5	180 $\pm$ 4	-15%	3 $\pm$ 0	10 $\pm$ 2	233%	52 $\pm$ 2	82 $\pm$ 4	58%
	10		949 $\pm$ 15	843 $\pm$ 16	-11%	18 $\pm$ 4	91 $\pm$ 13	405%	51 $\pm$ 3	86 $\pm$ 5	69%
	11		460 $\pm$ 0	375 $\pm$ 6	-18%	4 $\pm$ 1	12 $\pm$ 2	200%	61 $\pm$ 3	94 $\pm$ 10	54%
	Mean $\pm$ SE		15 $\pm$ 2%, 2P < 0.05		279 $\pm$ 64%, 2P < 0.05		60 $\pm$ 4%, 2P < 0.01				
	9	With	238 $\pm$ 1	179 $\pm$ 1	-25%				74 $\pm$ 2	111 $\pm$ 5	50%
11	844 $\pm$ 32		701 $\pm$ 19	-17%				62 $\pm$ 3	88 $\pm$ 3	42%	
Mean $\pm$ SE		268 $\pm$ 6	212 $\pm$ 9	-21%				68 $\pm$ 1	93 $\pm$ 1	37%	
Mean $\pm$ SE		21 $\pm$ 2%, 2P < 0.05					43 $\pm$ 4%, 2P < 0.01				

\*Before: indicates mean  $\pm$  SEM of levels in 1-min effluent fractions during the 5 min preceding the increase in glucose concentrations.

†During: indicates mean  $\pm$  SEM of hormone concentrations in the 15 1-min effluent fractions during perfusion with 11 mM glucose.

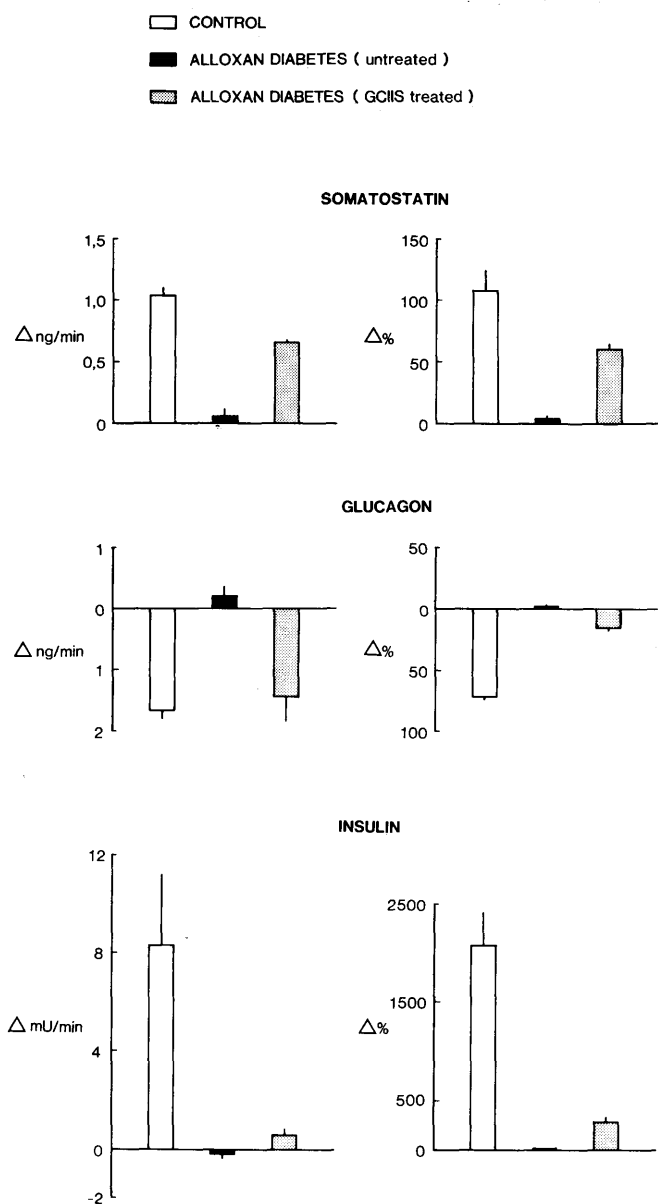
The percentage variations ( $\Delta\%$ ) is calculated from these mean levels.

taneous elevation of insulin and glucose in pancreas of untreated alloxan-diabetic dogs so as to simulate the glucose-induced rise in insulin levels of nondiabetic dogs could reproduce the normal pattern of glucose-induced stimulation of somatostatin and suppression of glucagon. A short-term infusion of exogenous insulin in vitro at a concentration thought to mimic the insulin level in the extracellular space of normal islets at high glucose was, however, not able to revive D- and A-cell responses (Figure 1). It could be argued that a 15-min insulin infusion in vitro was too short-lived. However, in STZ diabetes, a 75-min infusion of exogenous insulin was likewise without any effect.<sup>5</sup> Furthermore, insulin administration in vitro has minor or no effect on somatostatin<sup>5,19,20</sup> or glucagon secretion from the normal pancreas.<sup>5,17</sup> Thus, it is not loss of local alterations in insulin levels within the islet or lack of insulin surrounding the pancreatic islet cells per se that is responsible for the loss of glucose-sensing function of the D- and A-cells in insulin-deficient diabetes.

It was therefore obvious that the abnormality might be secondary to the diabetic metabolic aberrations. In this context, we have previously tested whether 1–3 days of conventional insulin treatment of STZ-diabetic dogs might resolve the islet cell insensitivity to glucose. No effect of this insulin treatment occurred (K. Hermansen and H. Ørskov, unpublished results), but the blood sugar levels in these dogs were not entirely normalized. Complete normalization of the blood glucose level in alloxan diabetes may be obtained by the artificial pancreas. As seen in Figures 6 and 7, the increment in perfusate glucose in these Biostator-treated animals is now able to induce a stimulation of somatostatin and suppression of glucagon, i.e., normal per-

tubations. Trimble and co-workers<sup>7</sup> found also a restoration of the pancreatic D-cell responses to glucose in STZ-diabetic rats successfully treated for 2–3 wk with a conventional insulin regimen. Although the D- and A-cell responses to glucose in the alloxan-diabetic dogs treated for 24 h by the artificial pancreas attained quantitatively normal increments and decrements, they did not reach normal percentage changes because the prestimulation levels were higher than in normal dogs. So the secretory machinery of all of the pancreatic D- and A-cells is still functionally damaged in some respects, despite the partially restored glucose sensitivity.

The possibility that the diabetogenic agents alloxan and STZ may exert some more direct contributory effect on islet cells has, however, not been totally erased from the scenario. In favor of such a proposal, it could be argued that Goto et al.<sup>10</sup> recently demonstrated an increase in basal somatostatin secretion in response to alloxan infusion in vitro and found that the D-cell responsiveness was subdued after alloxan and STZ infusions in vitro. It should be noted, however, that the latter dysfunction represented a D-cell insensitivity to all applied stimuli<sup>10</sup> rather than a selective impairment to glucose.<sup>5–7,18</sup> Furthermore, the findings in starvation diabetes are not consistent with the view that the D-cell abnormalities are caused by a cytotoxic effect of alloxan or STZ on the D-cell.<sup>21,22</sup> Thus, the pancreatic D-cells of 72-h starved dogs exhibit similar, though less pronounced, abnormalities, i.e., a diminished responsiveness to glucose and D-glyceraldehyde.<sup>21</sup> The present demonstration of normalization of glucose sensitivity by normalization of blood glucose also goes against a direct alloxan-induced permanent glucose receptor lesion.



**FIGURE 8.** Comparison of the average changes in islet hormone output to a 15-min change in extracellular glucose from 1.3 to 11 mM in normal (N = 4), untreated (N = 4), and insulin-treated alloxan-diabetic dogs (N = 3). Data showing changes in absolute terms are depicted at the left; and data showing percentage changes are depicted at the right. The data are shown as mean ± SEM.

In conclusion, the pancreatic D- and A-cells appear to be insensitive to fluctuations in glucose concentrations in insulinopenic diabetes in animals. It is possible that a functional defect common to the D- and A-cells may be operative in experimental diabetes. The restoration of glucose sensitivity of the pancreatic D- and A-cells by insulin treatment may occur as an insulin-induced correction of abnormal circulating levels of certain metabolites and/or hormones. The altered islet cell function in diabetes is related to the metabolic abnormalities rather than to the actual insulin concentration within the pancreas.

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