SUMMARY
We have proposed that chronic hyperglycemia causes the abnormal glucose influence on arginine-stimulated insulin secretion in the neonatal streptozocin (STZ) rat model of NIDDM and therefore studied the effect of 24 h of mild insulin-induced hypoglycemia on this defect. Ultralente insulin, 20 U/kg, was given at 9 a.m. and 10 U/kg at 5 p.m., and insulin and glucagon secretion were then studied the next morning using the in vitro isolated, perfused pancreas. The fed plasma glucose concentrations decreased in the STZ rats from 191 ± 13 to 101 ± 9 mg/dl and from 133 ± 4 to 99 ± 8 mg/dl in the controls.

As expected, 10 mM arginine caused a trivial insulin response at 2.8 mM glucose in the treated and untreated control groups compared with the marked one at 16.7 mM. The response to arginine at 2.8 mM glucose in the untreated STZ rats, however, was strikingly elevated (7.65 ± 2.29 versus 0.41 ± 0.16 ng/ml in the untreated controls) and it was not potentiated by the high glucose background, but the result at 2.8 mM glucose in the treated STZ rats was similar to that of the treated controls (0.46 ± 0.12 versus 0.16 ± 0.03 ng/ml). A return of glucose influence on IBMX-stimulated insulin secretion was also noted. Glucose-induced insulin release, however, was not restored in the treated STZ rats, but it was markedly suppressed in the controls by the insulin treatment.

Glucose influence on the glucagon response to arginine was maintained in the STZ model even though the glucagon release to a lowered glucose concentration was lost.

These data suggest that chronic hyperglycemia causes the abnormal glucose influence on arginine-stimulated insulin release in the STZ model of diabetes.

MATERIALS AND METHODS
Neonatal streptozocin (STZ) rat model. Two-day-old male, Sprague-Dawley pups received 90 mg/kg STZ intraperitoneally (i.p.) (Upjohn Company, Kalamazoo, Michigan) and controls received the diluent, 7 mM sodium citrate in 0.9% NaCl, pH 4.5. At 4 days of age, blood was obtained by cardiac puncture and the plasma glucose concentration measured using a Beckman Glucose Analyser II (Beckman Company, Brea, California). The STZ pups were subsequently followed if that value was >275 mg/dl. At 24 days of age, the animals were weaned and then received standard laboratory chow ad libitum. The fed plasma glucose concentration was measured at 8 wk of age in blood obtained...
by tail snipping, and the STZ rats were then studied with littermate controls at 8–13 wk of age if that value was >180 mg/dl.

**Insulin therapy.** The rats were weighed and then Ultralente insulin U-100 (Squibb Company, Princeton, New Jersey) 20 U/kg was given subcutaneously (s.c.) at 9 a.m. and 10 U/kg at 5 p.m. using a 0.5-ml Lo-dose insulin syringe (Becton-Dickinson Company, Rochelle Park, New Jersey). Food and water were available as desired. Blood was obtained for plasma glucose measurements at 9 a.m., 1 p.m., and 5 p.m. on the day of treatment, and at 9 a.m. the next morning in both treated and untreated STZ and control rats. Insulin and glucagon secretion were then studied with the in vitro isolated, perfused pancreas so that the duration of the insulin treatment was approximately 24 h.

**In vitro isolated, perfused pancreas.** This technique has been described previously. The perfusate was a modified Krebs-Ringer bicarbonate solution to which 4% dextran T70 (Sigma Company, St. Louis, Missouri), 2 mM calcium, and 0.2% bovine serum albumin fraction V (Sigma) were added. It was then equilibrated with 95% O2 and 5% CO2 and then subsequently passed through an artificial lung immediately before delivery to the animal to assure adequate oxygenation. Glucose was added to the desired concentration and the pH adjusted to 7.4. It was then placed in a reservoir maintained at 37–40°C by a water bath. The flow was nonrecirculating and the rate was controlled by a Polystaltic pump (Buchler Company, Fort Lee, New Jersey) so that the portal vein flow averaged 2.2 ml/min. Arginine (Eastman Company, Rochester, New York) or 3-isobutyl-1-methylxanthine (IBMX) (Sigma) was added to a second reservoir so that the flow could be switched from one to the other without evident lag or change in rate. The glucose concentration was increased by means of a sidearm syringe driven by a Harvard pump (Harvard Apparatus, Millis, Massachusetts). After the surgery, the animal was placed in a chamber maintained at 37°C, and the samples were collected (after a 20-min equilibration period) at predetermined points into chilled tubes containing 1 mg EDTA, and were kept on ice until being stored at –20°C. The perfusion protocols are depicted at the top of Figures 1, 2, and 3.

**Radioimmunoassay.** The insulin assay employed charcoal separation and rat insulin standards (Novo Research Institute, Copenhagen, Denmark). The glucagon concentrations were measured with an established radioimmunoassay using 30K or 04A antiserum (purchased from Dr. Roger Unger, Dallas, Texas).

**TABLE 1**

<table>
<thead>
<tr>
<th>Animals (N)</th>
<th>Fed plasma glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 a.m.</td>
</tr>
<tr>
<td>STZ (9)</td>
<td>191 ± 27</td>
</tr>
<tr>
<td>STZ-insulin (15)</td>
<td>191 ± 15</td>
</tr>
<tr>
<td>Control (13)</td>
<td>142 ± 5</td>
</tr>
<tr>
<td>Control-insulin (10)</td>
<td>124 ± 3</td>
</tr>
</tbody>
</table>

Data are combined for the different experiments and are expressed as mean ± SEM.

**RESULTS**

**Plasma glucose concentration during insulin treatment.**

The plasma glucose concentrations of the different groups measured at 0, 4, 8, and 24 h after the initial insulin injection...
The untreated STZ rats also released very low amounts of insulin to 2.8 mM glucose, but the addition of arginine caused a striking insulin response that peaked at 10.9 ± 3.56 ng/ml as compared with only 0.87 ± 0.25 in the untreated controls (2P < 0.001). The 16.7 mM glucose caused no increase in the insulin secretory rate, but arginine again caused a rapid, marked response that was very similar to that at 2.8 mM. Therefore, the marked modulating effect that glucose normally has on arginine-stimulated insulin secretion was absent in the untreated STZ rats. On the other hand, in the treated STZ rats, the response to arginine at 2.8 mM glucose was now similar to that of the controls, so that the glucose influence on arginine-stimulated insulin secretion was returned in the STZ rats by the insulin treatment. However, the response to 16.7 mM glucose alone was not restored.

Effect of insulin treatment on the insulin responses to glucose and IBMX. To determine if the mild hypoglycemia caused by the treatment regimen was inhibiting glucose-induced insulin secretion, and to study its effects on another nonglucose agent, IBMX, the protocol in Figure 2 was studied. The mean insulin concentrations for the different perfusate conditions are shown in Table 2.

As expected, the baseline insulin concentration caused by 27.8 mM glucose was very high in the untreated controls and it fell rapidly when the glucose was reduced to 2.8 mM. The reintroduction of 27.8 mM glucose caused a marked biphasic insulin response that peaked at 15.4 ± 4.50 ng/ml. The addition of 0.25 mM IBMX to 2.8 mM glucose caused a moderate rise in the insulin concentration, but the change to 27.8 mM produced a marked increase that averaged more than 16 times the previous level. On the other hand, the insulin-treated controls responded poorly to 27.8 mM glucose. Their baseline insulin level was only 17% of that of the untreated controls and their response to the reintroduction of the high glucose only peaked at 1.83 ± 0.62 ng/ml. The glucose increase from 2.8 to 27.8 mM markedly potentiated the response to IBMX even though that at 27.8 mM was lower than in the non-insulin-treated group.

The untreated STZ rats released very little insulin when 27.8 mM glucose was infused, but a paradoxical increase was seen when the glucose was reduced. The addition of IBMX to 2.8 mM glucose caused a marked response that was much greater than that in the controls. The higher glucose, however, produced only a minor increment over this, so that glucose regulation of IBMX-stimulated insulin secretion was also impaired. The response to 27.8 mM glucose was not improved in the insulin-treated STZ rats, but the paradoxical response to the lowered glucose level was abolished. The release to the IBMX at 2.8 mM glucose was now

**TABLE 2**

<table>
<thead>
<tr>
<th>Animals (N)</th>
<th>2.8 mM Glucose</th>
<th>2.8 mM Glucose + 10 mM arginine*</th>
<th>16.7 mM Glucose</th>
<th>16.7 mM Glucose + 10 mM arginine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>STZ (5)</td>
<td>0.74 ± 0.26</td>
<td>7.65 ± 2.29</td>
<td>0.84 ± 0.13</td>
<td>7.78 ± 1.22</td>
</tr>
<tr>
<td>STZ-insulin (5)</td>
<td>0.14 ± 0.05</td>
<td>0.41 ± 0.16</td>
<td>0.54 ± 0.09</td>
<td>5.04 ± 1.14</td>
</tr>
<tr>
<td>2P</td>
<td>0.02</td>
<td>2.96 ± 0.75</td>
<td>12.1 ± 3.99</td>
<td>NS</td>
</tr>
<tr>
<td>Control (5)</td>
<td>0.35 ± 0.20</td>
<td>0.46 ± 0.12</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Control-insulin (5)</td>
<td>0.12 ± 0.04</td>
<td>0.16 ± 0.03</td>
<td>2.78 ± 0.92</td>
<td>10.7 ± 1.66</td>
</tr>
<tr>
<td>2P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Insulin concentration is expressed as mean ± SEM

*Mean insulin concentration adjusted to represent the incremental response to arginine; see text for further information.

†Statistical significance determined using the unpaired, two-tailed Student t-test.
FIGURE 2. Effect of 24 h of insulin treatment on insulin response to 27.8 mM glucose and 0.25 mM IBMX in rats that received STZ as neonates and controls using the in vitro isolated, perfused pancreas. The data, obtained sequentially, are depicted with different insulin concentration scales for the responses to the 27.8 mM glucose and the IBMX. *P < 0.05.

similar to that of the controls, and the ability of glucose to potentiate that response was now evident, for that at 27.8 mM was almost five times greater than that at the low glucose background concentration.

Effect of insulin treatment on the insulin secretion after a lowered glucose concentration. To examine the effect of the insulin treatment on insulin release after a reduction in the perfusate glucose concentration, the protocol in Figure 3 was studied. The mean insulin concentrations caused by 11.1 and 2.8 mM glucose are listed in Table 3.

The insulin-treated control rats responded poorly to 11.1 mM glucose, but 2.8 mM glucose still caused a rapid fall in the insulin concentration to a level that averaged 23% of the baseline. The insulin-treated STZ rats had a very low baseline
level that transiently increased after the glucose reduction, but the absolute rise was very small, approaching the sensitivity of the assay. The secretory rate then fell to a level similar to that of the controls.

**Effect of insulin treatment on glucose regulation of arginine-stimulated glucagon secretion.** The adjusted mean glucagon concentrations caused by arginine at 2.8 mM glucose are compared with those at 16.7 mM for the different groups in Figure 4. In the untreated controls, the response at 16.7 mM was only 36% of that at 2.8 mM, showing that the glucose concentration also normally modulates the glucagon release to arginine. The insulin-treated controls behaved similarly, except for a significantly lower response at 16.7 mM. The untreated STZ rats maintained this inhibitory effect of the high glucose on the glucagon release, for their output at 16.7 mM was only 40% of that at 2.8 mM. The results in the insulin-treated STZ rats were almost identical. Therefore, glucagon regulation of arginine-stimulated glucagon secretion was intact in the STZ model.

**Effect of insulin treatment on the glucagon responses to a reduction in the glucose concentration.** The protocol and results are shown in Figure 5. The glucagon concentration of the untreated controls rose, after the reduction of glucose, to a level of 90 ± 12 pg/ml that was greater than the baseline of 43 ± 13 pg/ml (2P < 0.05) and then it gradually decreased when 2.8 mM glucose was reintroduced. However, in the insulin-treated controls, the glucagon response to the lowered glucose concentration was lost. The glucagon release to 2.8 mM glucose in the untreated STZ rats was similar to that of the controls (47 ± 12 versus 43 ± 13 pg/ml). However, the glucose reduction did not cause a glucagon response in either the treated or untreated STZ rats.

A similar loss of the ability of a lowered glucose concentration to stimulate glucagon release in insulin-treated control rats is found on examination of the glucagon results obtained from the protocol in Figure 3. The mean glucagon concentrations released at 11.1 and 2.8 mM glucose in insulin-treated control and STZ rats are shown in Table 3. The glucagon concentration did not increase significantly in either group after the glucose reduction.

**DISCUSSION**

Many agents besides glucose cause insulin secretion, but the response to some of them is not independent of glucose; instead, it is modulated by the background concentration at which they are given. An increase in glucose tends to potentiate the response while a decrease suppresses it. This regulatory role is clearly shown in the control rats of the present study using the mediators arginine and IBMX.

The neonatal STZ rat model of NIDDM is characterized by an insensitivity of the B-cell to glucose; specifically, glucose-induced insulin secretion is lost. Also, if the normal effect of glucose on arginine-stimulated insulin secretion were merely additive to the arginine response, one might expect in this model that it would be depressed at all glucose levels tested. Instead, near-maximal release occurs at even trivial glucose concentrations. This finding suggested to us that chronic exposure of these B-cells to elevated plasma glucose concentrations may have lead to a "potentiated" state, so that exposure to arginine even in the absence of glucose caused a marked response. We hypothesized that a lowering of the plasma glucose might reverse this potentiation and restore the modulating effect of glucose on the B-cell.

The results of the present study support our concept, for after 24 h of mild hypoglycemia, the response to arginine in the STZ rats at 2.8 mM glucose was now similar to that of the controls, while that at the high glucose background remained intact. A similar effect was demonstrated on IBMX-
this conclusion has come from recent observations in our laboratory showing that normal rats made hyperglycemic for 4 wk by glucose infusions also respond as well to arginine stimulation as at high ones (unpublished data).

Nonetheless, the modulating effect of glucose on the B-cell secretion might have been inhibited in the STZ rats as well, for the insulin concentration at 27.8 mM glucose was decreased from 0.92 ± 0.30 to 0.22 ± 0.09 ng/ml (2P < 0.06) in the treated group, and the level at 11.1 mM was reduced from 0.64 ± 0.18 to 0.22 ± 0.04 ng/ml (2P < 0.08). The paradoxical increase in insulin release noted in the STZ rats after a glucose reduction is also almost abolished by insulin treatment. Finally, Frankel et al.20 have documented suppression of insulin release in mildly diabetic Chinese hamsters after 4 wk of insulin treatment. While the cause of the decrease in insulin release is not entirely clear, suppression by the insulin administration itself or by a catecholamine response to the mild hypoglycemia are two possibilities. As such, the significance of the failure to restore glucose-induced insulin secretion in STZ rats is unknown. It is interesting that we have found, in STZ rats, that the response to glucose is lost at 4 wk of age, at a time when their plasma glucose concentration is barely abnormal.27 One explanation may be that the glucose range in which B-cell function is normal may be very narrow, and that small chronic increases or decreases lead to marked reduction in responsiveness to an acute glucose challenge.

Glucose is also known to influence the glucagon response to arginine22,23 in a way opposite to the B-cell, so that a glucose increase inhibits the glucagon release. This effect of glucose was clearly shown in the control rats in the present study. However, this glucose influence remained intact in the STZ model, for the response at 16.7 mM was only 40% of that at 2.8 mM, which compares to the 36% noted in the untreated controls. This result is particularly interesting, for Hollander et al.24 have shown, in a group of mild NIDDM patients, that glucose modulation of arginine-stimulated insulin secretion was severely impaired while that on the glucagon response remained intact. This finding contrasts with the abnormal direct effect that glucose has on glucagon secretion in this model.6,19 for neither an increase nor a decrease in the glucagon concentration caused a glucagon response. The insulin-treated STZ rats behaved similarly. Surprisingly, the insulin-treated control rats also had lost the influence of glucose on glucagon release, for the expected rise after the glucose drop from 27.8 to 2.8 mM was absent. We have previously shown that antecedent hyperglycemia can suppress the A-cell response to subsequent hypoglycemia,8 so a second protocol was tested in which the glucose was only decreased from 11.1 to 2.8 mM. However, the glucagon concentration again failed to rise. It is well established that exogenous insulin acutely suppresses glucagon release,25 but if in vivo hypoglycemia occurs, a glucagon response can break through this inhibition. The chronic effects of insulin treatment and mild hypoglycemia on the A-cell, however, are not well established. It may be that a chronically maintained narrow normal glucose range may also be a prerequisite for normal A-cell function, and that deviations in either direction can lead to abnormalities.

In summary, glucose regulation of arginine-stimulated insulin secretion is abnormal in the neonatal STZ rat model of NIDDM patients, that glucose modulation of arginine-stimulated insulin secretion was severely impaired while that on the glucagon response remained intact. This finding contrasts with the abnormal direct effect that glucose has on glucagon secretion in this model.6,19 for neither an increase nor a decrease in the glucagon concentration caused a glucagon response. The insulin-treated STZ rats behaved similarly. Surprisingly, the insulin-treated control rats also had lost the influence of glucose on glucagon release, for the expected rise after the glucose drop from 27.8 to 2.8 mM was absent. We have previously shown that antecedent hyperglycemia can suppress the A-cell response to subsequent hypoglycemia,8 so a second protocol was tested in which the glucose was only decreased from 11.1 to 2.8 mM. However, the glucagon concentration again failed to rise. It is well established that exogenous insulin acutely suppresses glucagon release,25 but if in vivo hypoglycemia occurs, a glucagon response can break through this inhibition. The chronic effects of insulin treatment and mild hypoglycemia on the A-cell, however, are not well established. It may be that a chronically maintained narrow normal glucose range may also be a prerequisite for normal A-cell function, and that deviations in either direction can lead to abnormalities.

In summary, glucose regulation of arginine-stimulated insulin secretion is abnormal in the neonatal STZ rat model of diabetes.
REVERSAL OF ABNORMAL INSULIN SECRETION

NIDDM and is manifested as a hyperresponse at low glucose concentrations. This defect is reversed by 24 h of mild hyperglycemia, suggesting that chronic hyperglycemia is its cause. Glucose-induced insulin secretion was not restored, and it was markedly impaired by the insulin treatment in the controls. A glucagon response to hyperglycemia was also not restored in the STZ rats, and it similarly was lost in the treated control rats. One explanation may be that normal A- and B-cell functions may be dependent on a maintained narrow normal glucose range, and that deviations in either upward or downward direction severely impair insulin and glucagon secretion.

ACKNOWLEDGMENTS

The authors thank C. Forther and L. Braunstein for excellent technical assistance and J. Reynolds for preparation of the manuscript.

This work was supported by grant AM-20349 from the NIH.

REFERENCES