# Regulation of α-Cell Function by the β-Cell During Hypoglycemia in Wistar Rats: the "Switch-off" Hypothesis

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**The glucagon response is the first line of defense against hypoglycemia and is lost in insulin-dependent diabetes. The β-cell "switch-off" hypothesis proposes that a sudden cessation of insulin secretion from β-cells into the portal circulation of the islet during hypoglycemia is a necessary signal for the glucagon response from downstream α-cells. Although indirect evidence exists to support this hypothesis, it has not been directly tested in vivo by provision and then discontinuation of regional reinsulinization of α-cells at the time of a hypoglycemic challenge. We studied streptozotocin (STZ)-induced diabetic Wistar rats that had no glucagon response to a hypoglycemic challenge. We reestablished insulin regulation of the α-cell by regionally infusing insulin (0.025 μU/min) directly into the superior pancreaticoduodenal artery (SPDa) of STZ-administered rats at an infusion rate that did not alter systemic venous glucose levels. SPDa insulin infusion was switched off simultaneously when blood glucose fell to <60 mg/dl after a jugular venous insulin injection. This maneuver restored the glucagon response to hypoglycemia (peak change within 5–10 min = 326 ± 98 pg/ml, $P < 0.05$; and peak change within 15–20 min = 564 ± 148 pg/ml, $P < 0.01$). No response was observed when the SPDa insulin infusion was not turned off (peak change within 5–10 min = 44 ± 85 pg/ml, $P$ = NS; and peak change within 15–20 min = 67 ± 97 pg/ml, $P$ = NS) or when saline instead of insulin was infused and then switched off (peak change within 5–10 min = −44 ± 108 pg/ml, $P$ = NS; and peak change within 15–20 min = −13 ± 43 pg/ml, $P$ = NS). No responses were observed during euglycemia (peak change within 5–10 min = 48 ± 35 pg/ml, $P$ = NS; and peak change within 15–20 min = 259 ± 129 pg/ml, $P$ = NS) or hyperglycemia (peak change within 5–10 min = 49 ± 62 pg/ml, $P$ = NS; and peak change within 15–20 min = 138 ± 87 pg/ml, $P$ = NS). Thus, the glucagon response to hypoglycemia that was absent in rats made diabetic by STZ was restored by regional infusion and then discontinuation of insulin. These data provide direct in vivo support for the β-cell "switch-off" hypothesis and indicate that the α-cell is not intrinsically abnormal in insulin-dependent diabetes because of STZ-induced destruction of β-cells. *Diabetes* 53:1482–1487, 2004**

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SPDa, superior pancreaticoduodenal artery; STZ, streptozotocin.



Glucagon is the key hormone released in response to hypoglycemia (1–4). It counterregulates the fall in blood glucose through stimulation of hepatic glycogenolysis (5,6). People with type 1 diabetes lose the glucagon response to hypoglycemia, although α-cell responses to other stimuli are retained (7,8). Loss of the glucagon response is often associated with a reduced epinephrine response and symptom unawareness to hypoglycemia (9,10). These combined defects render hypoglycemia a major obstacle to the completely successful management of diabetes with exogenous insulin and thereby limit the possibility of preventing long-term diabetes complications (11,12).

Regulation of α-cell function by insulin secreted into the portal circulation of the islet from upstream β-cells was first proposed in 1971 by Samols et al. (13). More recently, this concept has been applied to the clinical problem of hypoglycemia and has been used by Cryer et al. (14) to formulate the intraislet insulin hypothesis, which envisions a β-cell switch off of insulin secretion as a key mechanism for the glucagon response to hypoglycemia. However, no previously reported research has directly tested this hypothesis in a diabetic model by regional provision of exogenous insulin to the α-cell and then discontinuation of the insulin signal at the time of hypoglycemia.

We formulated three questions to directly test the β-cell switch-off hypothesis. *1*) Are intact β-cells required for the glucagon response to hypoglycemia? *2*) If so, will restoration and then discontinuation of regional insulinization of the α-cell return the glucagon response to hypoglycemia? *3*) If so, does the α-cell response to switching off exogenous insulin secretion occur only in the setting of hypoglycemia or also during euglycemia and hyperglycemia? To answer these questions, we *1*) compared the glucagon response to hypoglycemia in normal rats with that in streptozotocin (STZ)-administered rats, *2*) infused insulin into the pancreaticoduodenal artery of STZ-administered rats and switched off the infusion when the animals became hypoglycemic because of insulin pulses given via the jugular vein, and *3*) examined the glucagon response in STZ-administered animals when glucose levels were <60,

60–160, and >160 mg/dl at the time of pancreatic artery insulin switch off.

## RESEARCH DESIGN AND METHODS

Male Wistar rats with and without right jugular vein cannulation and initially weighing 200–250 g were purchased from Charles River Laboratories. Animals were placed in rooms with a 12-h light/dark cycle and constant temperature and given free access to food and water. All experiments were approved by the Pacific Northwest Research Institute's Institutional Animal Care and Use Committee. Animals were randomly divided into diabetic and control groups. Diabetes was induced by an intraperitoneal injection of 80 mg/kg STZ (Sigma, St. Louis, MO) (15,16). After STZ injection, blood glucose was measured every other day. Rats were considered diabetic when blood glucose exceeded 350 mg/dl on two sequential measurements. Animals that did not develop diabetes by 1 day were given the same dose of STZ again. Insulin pellets (Linshin Canada, Scarborough, ON, Canada) delivering ~1 unit/24 h were inserted under the skin. Blood glucose levels in insulin-treated diabetic rats were 100–250 mg/dl. Animals that were diabetic for at least 2 weeks were used for the experiments, at which time they weighed between 350 and 400 g.

**Surgical procedure.** On the day of the study, animals were anesthetized with an intraperitoneal injection of 80 mg/kg ketamine and 10 mg/kg xylazine. Anesthesia was maintained by further injection of small doses as necessary. The necks and abdomens were shaved. In animals not already cannulated at the time of purchase, right jugular veins were identified and cannulated with PE50 polyethylene tubing (Becton Dickinson, Spark, MD) filled with heparin (500 units/ml), which was advanced to the superior vena cava. This cannula was used for intravenous insulin infusion and collection of blood samples. The upper abdomen was opened by a 3-cm vertical incision in the midline starting from the xiphoid process. The pancreas typically received its blood supply from branches of the splenic and superior and inferior pancreaticoduodenal arteries (17–19). The hepatic artery was isolated and punctured by a 25-gauge needle. A microcannula (0.008 mm I.D.; Biotime, Berkeley, CA) was inserted into the superior pancreaticoduodenal artery (SPDa) via the hepatic artery for infusion of insulin. After surgery, the open abdomen was superfused with warm saline and covered with foil to prevent drying. All animals were studied nonfasted.

**Insulin infusion protocols**

***Protocol 1.*** Normal and STZ-induced diabetic animals were studied awake and moving freely in their cages. Insulin (12 units/kg [in 12 units/ml solution]) was injected into the jugular vein, and samples were collected for blood glucose, C-peptide, and glucagon measurements. Glucose levels were measured within 2 min of sample collection. These animals were also given arginine (70 mg/kg) to assess α-cell responsiveness to a nonhypoglycemic stimulus.

***Protocol 2.*** After the surgical procedures described above, unconscious STZ-induced diabetic rats were rested for 30 min. Then, 0.5 units/kg insulin was injected into the jugular vein to decrease blood glucose by ~100 mg/dl. After two basal blood samples were collected, insulin (0.025 units/min), at an infusion rate that did not affect the systemic blood glucose level, or saline was infused into the SPDa. Ten minutes later, an insulin bolus (12 units/kg) was injected into the jugular vein to achieve hypoglycemia. When blood glucose was <60 mg/dl, the pancreatic artery insulin or saline control infusion was switched off and blood was sampled at 5, 10, 15, 20, 30, and 60 min. In some animals, the insulin infusion was not switched off but continued until the end of the experiment.

***Protocol 3.*** In this group of animals, the SPDa insulin infusion was conducted, but no jugular vein injection of insulin to cause hypoglycemia was given, so that the animals remained either euglycemic or hyperglycemic. The pancreatic artery insulin infusion was switched off and the blood was withdrawn at the same time points as in protocol 2.

**Assays.** Plasma glucose was measured immediately using a glucose analyzer II (Beckman, Fullerton, CA). Blood samples were collected into heparin-coated, ice-chilled tubes. A total of 1,000 IU/ml trasylol was added to prevent degradation of glucagon. Plasma C-peptide was measured using a rat C-peptide radioimmunoassay (Linco Research, St. Charles, MO). Plasma pancreatic glucagon was measured by an enzyme immunoassay kit (Yanaihara Institute, Shizuoka, Japan).

**Statistical analysis.** Data are presented as means ± SE. Results were analyzed using Wilcoxon's matched pair signed-rank test or ANOVA as appropriate. A $P$ value <0.05 was considered statistically significant.

## RESULTS

**Glucagon responses to hypoglycemia in control and STZ-induced diabetic rats.** Although the baseline values of blood glucose were significantly higher in diabetic rats before insulin injection, control and diabetic rats achieved similar levels of hypoglycemia (25 ± 3 and 30 ± 3 mg/dl, respectively) (Fig. 1). Hypoglycemia in control rats was counterregulated by 30 min (0 min = 25 ± 3; 30 min = 94 ± 31 mg/dl; $P < 0.05$). By 60 min, glucose levels in the control groups were significantly higher than those in the STZ-administered group (112 ± 7 vs. 44 ± 5 mg/dl, respectively; $n = 16$; $P < 0.001$). In normal rats, the fall in plasma glucose level was accompanied by significant decrements in plasma C-peptide (from 443 ± 64 to 76 ± 31 pmol/l at 0 vs. 30 min, $P < 0.01$). In STZ-administered animals, basal plasma C-peptide levels were low initially (60 ± 21 vs. 443 ± 64 pmol/l, STZ-administered animals vs. controls, $P < 0.001$) (Fig. 1) and remained low. The mean plasma glucagon levels in the basal state were comparable in the two groups (546 ± 63 vs. 651 ± 55 pg/ml, STZ-administered animals vs. controls, $P$ = NS). In normal rats, there was a rapid and marked increment of glucagon in response to hypoglycemia (0- to 15-min peak change = 261 ± 23 pg/ml; $n = 7$, $P < 0.001$) (Fig. 1). No glucagon response was observed in STZ-administered animals. Glucagon responses 3 min after intravenous arginine were intact in both groups (normal = 330 ± 90 pg/ml; diabetic = 500 ± 340 pg/ml, $P$ = NS).

**Glucagon responses to hypoglycemia after pancreatic artery insulin infusion switch off.** STZ-administered diabetic rats were divided into three groups: the insulin switch-off group (group A), the no insulin switch-off group (group B), and the saline control group (group C). All three groups achieved similar hypoglycemia (<60 mg/dl) (Fig. 2). Group A had an increase in glucose after the pancreatic artery insulin infusion was switched off (nadir = 41 ± 7, response = 128 ± 38 mg/dl, $n = 5$, $P < 0.05$) but groups B and C did not. Plasma C-peptide levels remained low in all three groups and confirmed major β-cell destruction (Fig. 2). Switching off the pancreatic artery insulin infusion restored the glucagon response to hypoglycemia in group A (peak change within 5–10 min = 326 ± 98 pg/ml, $P < 0.05$, and peak change within 15–20 min = 564 ± 148 pg/ml, $P < 0.01$) (Fig. 2). This response was greater than that observed in the control group in Fig. 1 (peak change = 564 ± 148 vs. 261 ± 23 pg/ml, $P < 0.05$). Group B, in which the SPDa insulin infusion was not turned off, and group C, the saline control group, had no glucagon responses to hypoglycemia (group B: peak change within 5–10 min = 44 ± 85 pg/ml, $P$ = NS, and peak change within 15–20 min = 67 ± 97, $P$ = NS; and group C: peak change within 5–10 min = −44 ± 108, $P$ = NS, and peak change within 15–20 min = −13 ± 43, $P$ = NS).

**Glucagon responses during euglycemia and hyperglycemia after SPDa insulin infusion switch off.** During euglycemia (60–160 mg/dl) or hyperglycemia (>160 mg/dl), no significant acute glucagon responses were observed in diabetic rats after SPDa insulin switch off (euglycemic group D: peak change within 5–10 min = 48 ± 35 pg/ml, $P$ = NS, and peak change within 15–20 min = 259 ± 129, $P$ = NS; and hyperglycemic group E: peak change within 5–10 min = 49 ± 62, $P$ = NS, and peak change within 15–20 min = 138 ± 87, $P$ = NS) (Fig. 3). A statistically significant linear correlation was found between the glucose nadir and the magnitude of

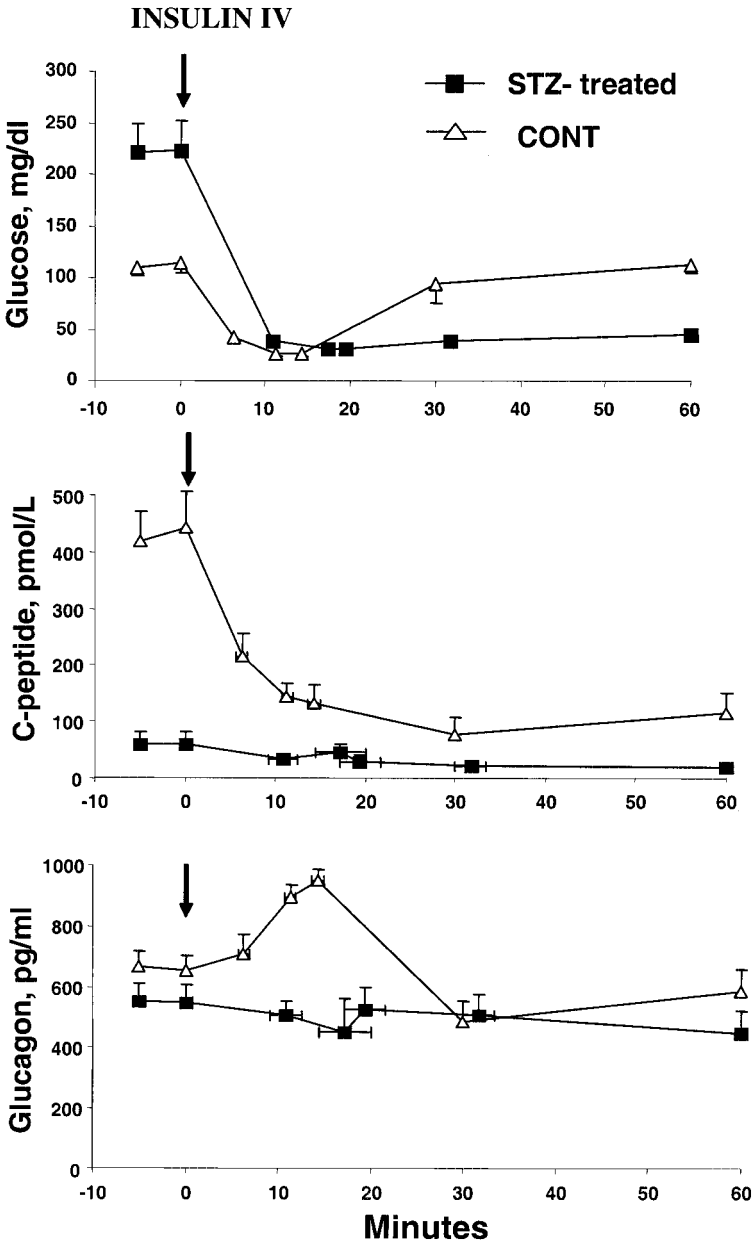


**FIG. 1. Comparison of responses to hypoglycemia in control and STZ-administered rats. Insulin was injected into the jugular vein. Samples were collected for blood glucose, C-peptide, and glucagon measurements. $n$ = 7 control animals, $n$ = 9 STZ-administered animals. The normal, but not the STZ-administered, rats had glucagon responses to hypoglycemia and experienced successful glucose counterregulation. CONT, control; IV, intravenous.**

the glucagon response when all three (hypoglycemic, $n = 5$; euglycemic, $n = 5$; and hyperglycemic, $n = 5$) SPDa insulin switch-off groups were examined as one combined group ($n = 15$; $r = 0.56$; $P < 0.05$).

## DISCUSSION

This study was designed to assess the β-cell "switch-off" hypothesis in vivo in the context of glucagon responses to hypoglycemia. We observed that destruction of β-cells by STZ in Wistar rats was accompanied by failure of the α-cell to release glucagon during hypoglycemia, although the α-cell response to arginine remained intact. Restoration of α-cell insulinization by exogenous insulin infusion into the pancreatic artery of STZ-administered diabetic rats, followed by switching off the insulin infusion when the animals became hypoglycemic because of an insulin bolus given via the jugular vein, was accompanied by restoration of the glucagon response. This effect was not seen if insulin was infused into the pancreatic artery but not switched off or if saline was infused instead of insulin. Restoration was also not seen if animals were euglycemic or hyperglycemic at the time of the SPDa insulin switch off.

Absence of glucagon responses during hypoglycemia caused by insulin therapy is a key feature of type 1 diabetes. The failure of this response greatly compromises the patient's ability to counterregulate hypoglycemia and can lead to severe clinical consequences. Gerich et al. (7) were the first to report that type 1 diabetic patients with no glucagon response to hypoglycemia still had intact glucagon responses when stimulated with intravenous arginine. This observation established that the glucagon secretory defect during hypoglycemia in diabetic patients is not due to a global defect in α-cell function but rather involves a specific defect in sensing hypoglycemia. Previous studies (20,21) emphasized the importance of the glucagon response during counterregulation of hypoglycemia in humans. Sjoberg et al. (22) found that diabetic subjects with

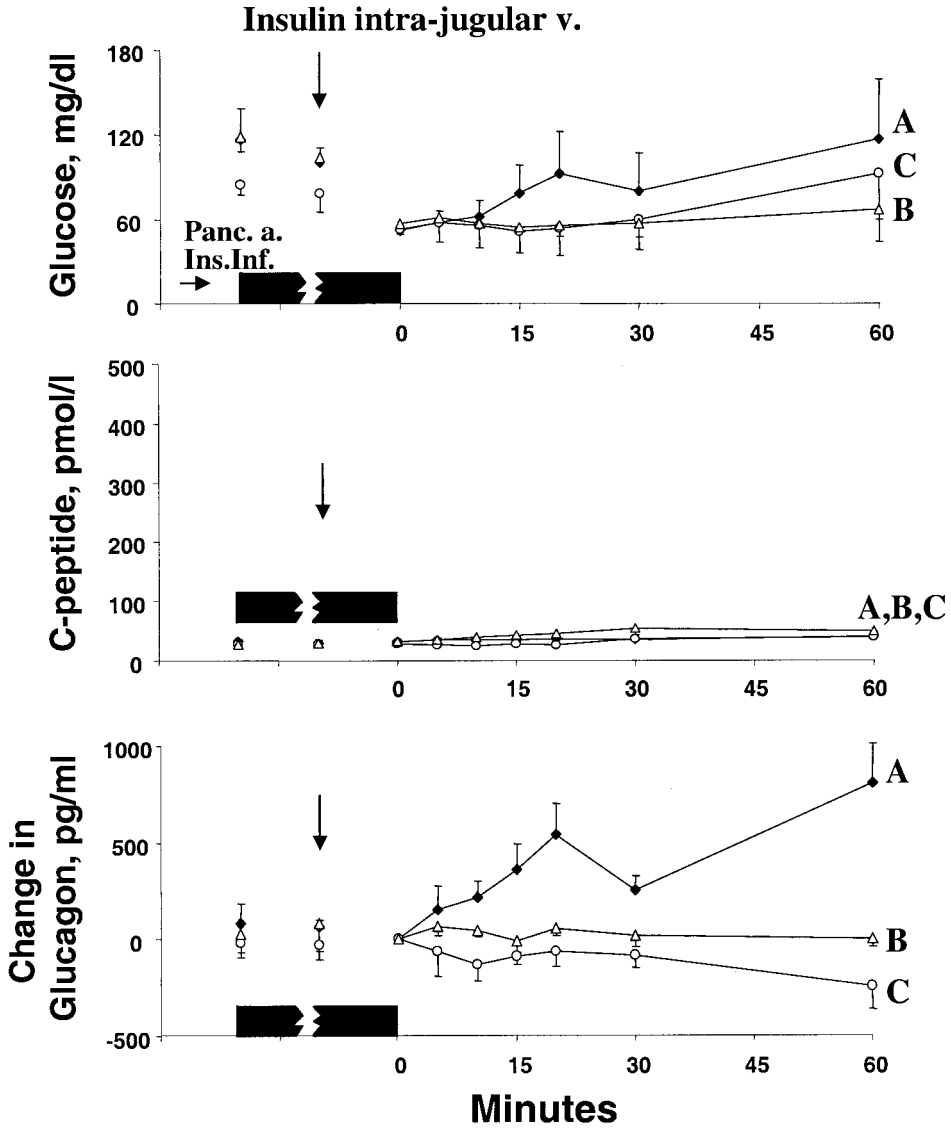


**FIG. 2. Effect of reestablishing an insulin switch-off signal on responses to hypoglycemia in STZ-administered rats. Insulin was injected into the jugular vein to decrease blood glucose to ~100 mg/dl. After baseline blood samples were collected, insulin (0.025 units/min) was infused into the SPDa, followed in 10 min by a 12-units/kg insulin bolus in the jugular vein to induce hypoglycemia. When blood glucose was <40 mg/dl, the pancreatic artery insulin infusion was switched off and blood was sampled for measurements of blood glucose (*top*), C-peptide (*middle*), and glucagon (*bottom*). Group A: SPDa insulin infused then switched off, $n = 5$; group B: SPDa insulin infused but not switched off, $n = 4$; group C: SPDa saline infused and then switched off, $n = 4$. Only the animals in which pancreatic artery insulin infusion was switched off had a glucagon response to hypoglycemia. Ins. Inf., insulin infusion.**

or without C-peptide levels in urine had no glucagon response to hypoglycemia. However, Fukuda et al. (23) reported earlier that glucagon responses were absent during insulin-induced hypoglycemia in diabetic patients who were plasma C-peptide negative but present in patients who were plasma C-peptide positive and suggested that it was the absence of β-cell function that might be causally related to defective α-cell dysfunction during hypoglycemia. Thereafter, several reports (24–28) appeared indicating that high concentrations of circulating insulin suppress the glucagon response to hypoglycemia in normal volunteers and diabetic subjects. Peacey et al. (29,30) observed decreased glucagon responses during hypoglycemia when normal subjects were treated with the β-cell agonist tolbutamide. These studies were conducted during a hypoglycemic clamp, during which glucose levels were maintained at the same level in drug-treated and nontreated subjects. The authors concluded that the decreased glucagon response during tolbutamide treatment indicates that enhanced β-cell secretion of insulin dampens the glucagon response to hypoglycemia. Similar studies were reported by Landstedt-Hallin et al. (31) and Banarer et al. (14). Segel et al. (32) reported that patients with type 2 diabetes with severe insulin insufficiency also had defective glucagon responses to hypoglycemia. Reasoning from these interrelationships between insulin secretion and glucagon secretion during hypoglycemia and based on earlier published evidence that decreased β-cell insulin secretion leads to increased glucagon secretion, Cryer et al. (14) have championed the hypothesis that defective glucagon secretion during hypoglycemia in diabetic patients might be due to the lack of a switch-off signal from the β-cell. This hypothesis had earlier been rejected by Bolli et al. (33), who examined glucagon responses during hypoglycemia under conditions of varying exogenous insulin and glucose levels in clamp studies in normal subjects. They found similar glucagon responses under all conditions and concluded that hypoglycemia is the primary signal for glucagon secretion independent of insulin levels. However, in those studies, endogenous β-cell secretion, as measured by C-peptide, consistently declined as glucose levels fell; consequently, they do not exclude a β-cell switch-off signal.

The many reports cited above are all consistent with the hypothesis that insulin from the β-cell flowing downstream in the portal circulation of the islet suppresses

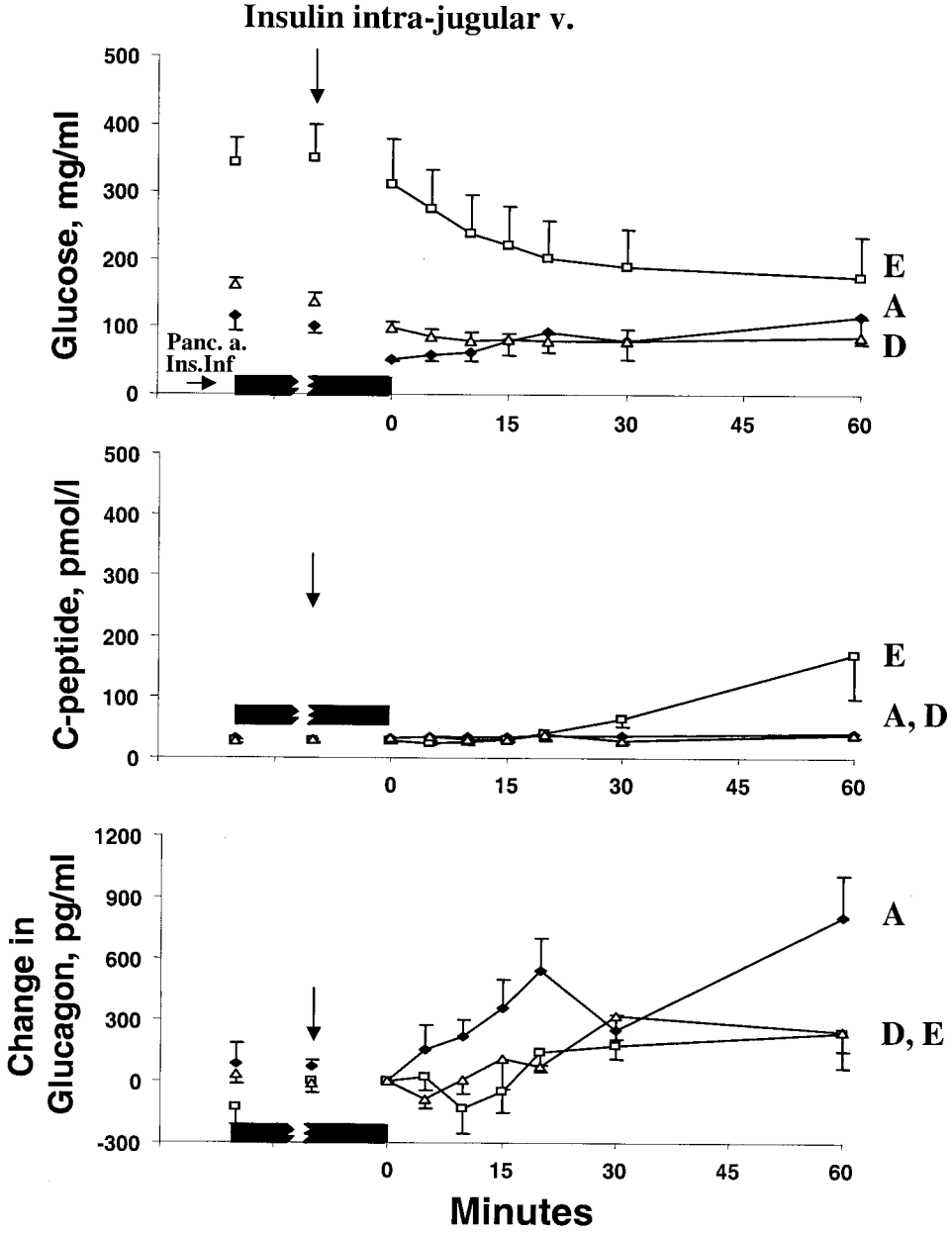


**FIG. 3. Comparison of glucagon responses to the insulin switch-off signal in STZ-administered rats that were or were not hypoglycemic. Euglycemic conditions (group D, $n = 5$) were defined as blood glucose = 60–160 mg/dl; hyperglycemic conditions (group E, $n = 5$) were defined as blood glucose >160 mg/dl. Group A data are from Fig. 2. Only the animals that were hypoglycemic had a glucagon response. Ins.Inf., insulin infusion; Panc. a., pancreatic artery.**

glucagon secretion. This was pointed out in early elegant studies by several groups of investigators (34–36). However, only recently has it been considered in the context of hypoglycemia, i.e., the decrease in insulin secretion caused by hypoglycemia is a necessary signal for an increase of glucagon secretion. The experiments reported in this article are novel because they demonstrate that reestablishing the switch-off signal restores glucagon responses to hypoglycemia in an animal model of type 1 diabetes. The normal responses in glucagon that we observed after this maneuver provide direct evidence in support of the switch-off hypothesis. The additional observations that this glucagon response is observed only during hypoglycemia and not during euglycemia or hyperglycemia underscore the fact that both the hypoglycemic signal and the insulin switch-off signal are required for the glucagon response. These conclusions are substantiated by Hope et al. (37), who report data from experiments using perifused islets isolated from normal rats and humans and from STZ-administered rats.


## ACKNOWLEDGMENTS

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