# Amelioration of Hypoglycemia Via Somatostatin Receptor Type 2 Antagonism in Recurrently Hypoglycemic Diabetic Rats

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Selective antagonism of somatostatin receptor type 2 (SSTR2) normalizes glucagon and corticosterone responses to hypoglycemic clamp in diabetic rats. The purpose of this study was to determine whether SSTR2 antagonism (SSTR2a) ameliorates hypoglycemia in response to overinsulinization in diabetic rats previously exposed to recurrent hypoglycemia. Streptozotocin diabetic rats ($n$ = 19), previously subjected to five hypoglycemia events over 3 days, received an insulin bolus (10 units/kg i.v.) plus insulin infusion (50 mU/kg/min i.v.) until hypoglycemia ensued (≤3.9 mmol/L) (experimental day 1 [Expt-D1]). The next day (Expt-D2), rats were allocated to receive either placebo treatment ($n$ = 7) or SSTR2a infusion (3,000 nmol/kg/min i.v., $n$ = 12) 60 min prior to the same insulin regimen. On Expt-D1, all rats developed hypoglycemia by ~90 min, while on Expt-D2, hypoglycemia was attenuated with SSTR2a treatment (nadir = 3.7 ± 0.3 vs. 2.7 ± 0.3 mmol/L in SSTR2a and controls, $P < 0.01$). Glucagon response to hypoglycemia on Expt-D2 deteriorated by 20-fold in the placebo group ($P < 0.001$) but improved in the SSTR2a group (threefold increase in area under the curve [AUC], $P < 0.001$). Corticosterone response deteriorated in the placebo-treated rats on Expt-D2 but increased twofold in the SSTR2a group. Catecholamine responses were not affected by SSTR2a. Thus, SSTR2 antagonism after recurrent hypoglycemia improves the glucagon and corticosterone responses and largely ameliorates insulin-induced hypoglycemia in diabetic rats. ***Diabetes*** **62:2215–2222, 2013**

The management of type 1 diabetes mellitus is impeded by the constant threat of hypoglycemia, caused by the inability to achieve physiological insulin replacement and because of a failure in the hormone counterregulation of hypoglycemia (1). Recurrent hypoglycemia increases the susceptibility to subsequent hypoglycemia, since it contributes to both defective hormone counterregulation and reduced symptom recognition (2). The reduction in symptom recognition for hypoglycemia has a profound impact on patient quality of life, and this population fears hypoglycemia more than long-term complications (3,4). The elevated risk of recurrent hypoglycemia, often precipitated by intensive insulin therapy, frequently necessitates a relaxation in management, which ultimately places the individual at risk for earlier complications (3). Currently, there are few prophylactic strategies that limit the risk of developing insulin-induced hypoglycemia (5), perhaps because the neuroendocrine mechanism(s) of impairment has yet to be fully elucidated. None of these treatments would be considered a preventative pharmacological approach.

With repeated exposure to hypoglycemia, there are impairments in the neuroendocrine and autonomic responses to subsequent hypoglycemia (6–9), perhaps because of defects in the regions of the central nervous system that detect and respond to hypoglycemia (1). In addition to numerous neuroendocrine deficiencies related to glucose sensing and blunted counterregulatory responses because of central deficiencies (7,10–14), elevation in circulating somatostatin levels in type 1 diabetes mellitus has long been thought to impair the counterregulatory response to insulin-induced hypoglycemia (15–20).

Somatostatin acts on various receptor subtypes (somatostatin receptor type [SSTR]1–5), being both a regulator of hormone secretion (typically inhibitory) and a neurotransmitter (21). With respect to glucose counterregulatory hormones, somatostatin release in the brain lowers pituitary growth hormone secretion indirectly via hypothalamic suppression of growth hormone–releasing hormone release and directly by acting on somatotrophs via SSTR2 and -5 (22). In the adrenal gland, somatostatin inhibits acetylcholine stimulated medullary catecholamine secretion and inhibits corticosteroid secretion predominantly via SSTR2 (23). In humans, somatostatin lowers pancreatic glucagon and insulin release through SSTR2 (24). In rats, somatostatin inhibits insulin secretion predominantly through SSTR5 (25) and glucagon secretion exclusively through SSTR2 (21).

Paradoxically, somatostatin concentrations are elevated at baseline and rise further during hypoglycemia in patients with type 1 diabetes mellitus who are on exogenous insulin (19). Various animal models of type 1 diabetes mellitus (7,17,18,26) and isolated islet studies in healthy rats (27) have demonstrated that elevations in somatostatin limit the glucagon response to hypoglycemia or arginine stimulation via SSTR2 activation. Since somatostatin also inhibits the release of all of the key hormones involved in glucose counterregulation (i.e., cortisol, growth hormone, catecholamines) (21,28), an elevation in somatostatin levels in type 1 diabetes mellitus may be one of the reasons why glucose counterregulation fails. Accordingly, the systemic administration of a somatostatin receptor agonist exacerbates severe hypoglycemia in

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patients with type 1 diabetes mellitus (29), likely because of reductions in glucose counterregulatory hormone levels to ensuing insulin-induced hypoglycemia. Thus, the use of a SSTR2 antagonist (SSTR2a) may be helpful in improving glucose counterregulation in this patient population. In support of this, we recently demonstrated that SSTR2a (PRL-2903) normalizes the glucagon and corticosterone responses to hypoglycemic clamp in diabetic rats (26). Since these were glucose clamp experiments, it was not possible to determine whether hypoglycemia could be prevented with SSTR2 antagonism. It is also unclear whether the improvement in the counterregulatory hormone response caused by SSTR2a would have favorable effects on glucoregulation in diabetes. In this present work, we tested the hypothesis that hypoglycemia can be prevented/attenuated with SSTR2 antagonism treatment in animals previously exposed to repeated hypoglycemic challenge by enhancing counterregulatory responses. We demonstrate here that the glucagon and corticosterone responses improve by SSTR2 antagonism and that the depth and duration of hypoglycemia are ameliorated in diabetic rats previously exposed to recurrent hypoglycemia.

## RESEARCH DESIGN AND METHODS

This was a repeated-measures randomized design study (placebo/placebo vs. placebo/SSTR2a) to test the effectiveness of SSTR2 antagonism on glucose and hormonal counterregulation during insulin-induced hypoglycemia in diabetic rats previously exposed to recurrent hypoglycemia. Nineteen male Sprague-Dawley rats (Charles River Laboratories, Saint-Constant, QC, Canada) with an initial body mass of 275–300 g were used. Rats were individually housed in opaque cages in a light- and temperature-controlled environment (12-h light:12-h dark cycle, 20–22°C) and fed ad libitum with chow (Harlan Laboratories, Madison, WI) with free access to food and water. After 1 week of experimenter handling and acclimatization, rats were given a single intraperitoneal streptozotocin (STZ) injection (65 mg/kg dissolved in 0.9% saline; Sigma) to induce diabetes. STZ-injected rats that did not become hyperglycemic within 48 h were excluded from the study. Morning (fed) glycemia (Ascencia Elite handheld glucometer; Bayer Canada, Etobicoke, ON, Canada), body mass, and food intake were measured daily. With use of aseptic technique, rats were anesthetized and catheterized with indwelling cannulae in the left carotid artery and right jugular vein 14 days after STZ injection. The cannulae were exteriorized, fed through a metal coil tether, and connected to a swivel system (rodent tether and swivel; Lomir Biomedical, Notre-Dame-de-Î Île Perrot, QC, Canada). This rodent tethering system allowed for manual, undisturbed blood sampling and infusions (arterial and venous catheters, respectively) and unrestricted movement of the rat while protecting the catheters. Catheters were flushed daily with heparinized (10 U.S. pharmacopeia units/mL) saline to ensure patency. Eighteen days after STZ injection, rats were subjected to recurrent hypoglycemia treatment over 3 days via a hyperinsulinemic-hypoglycemic clamp technique. (See below for details.) Twenty-one days after STZ injection, rats underwent a standardized 2-day back-to-back hypoglycemic challenge via insulin infusion either with or without SSTR2a. (See below for details.) All procedures were in accordance with the Canadian Council on Animal Care Standards and were approved by the animal care committee of the University of Toronto

**Recurrent hypoglycemia treatment.** Nineteen ($n$ = 19) rats were subjected to five episodes of recurrent hypoglycemia over 3 days using a modified hyperinsulinemia-hypoglycemic clamp technique. Rats were partially fasted overnight (10–15 g rat chow or ~25–40% ad libitum consumption with free access to 5% sucrose) prior to each day of recurrent hypoglycemia. On each morning of hypoglycemic challenge, basal blood glucose was measured at $t$ = 0 min and insulin (10 units/kg) was injected subcutaneously to induce hypoglycemia. Glucose infusions (50% dextrose) were given at a variable rate to clamp glycemia at a target hypoglycemia of 3.0 ± 0.5 mmol/L. Blood glucose was measured (Analox glucose analyzer, GMD-9D; Analox Instruments USA, Lunenburg, MA) in duplicate every 15 min for 180 min. During a rest period between 180 and 240 min, rats were given access to 5% sucrose water and intravenous glucose infusion to recover. At 240 min, rats again underwent a hypoglycemic challenge until 420 min. Food and sucrose water were fed to aid recovery after hypoglycemia treatment.

**Experimental days 1 and 2.** After this hypoglycemic conditioning period, each rat then underwent two additional experimental days of hypoglycemic challenge (i.e., with or without SSTR2a treatment), with measurements of their hormonal and glycemic responses. Rats were partially fasted overnight prior to each experimental day, as described above, to allow for standardization in food intake and preservation of liver glycogen stores. In the morning, rats were weighed, connected to venous infusion lines, and acclimated for 2 h prior to experimentation. Basal blood samples for glucose and hormones were taken at the start of the experiment ($t$ = −60 min) from freely moving, conscious rats with cannulae exteriorized outside of the cage. On experimental day 1 (Expt-D1), which served as the control day to measure the extent of counterregulatory failure caused by recurrent hypoglycemia, 0.9% saline infusion (1 mL/h) was started in all rats ($n$ = 19) after basal samples were obtained at $t$ = −60 min. Blood glucose levels were measured in duplicate using a glucose analyzer at times −60, −40, −20, and 0 min and every 10 min thereafter until 180 min. Blood samples for glucagon, catecholamines, and insulin were collected in chilled tubes containing EDTA (Sangon, Canada, Scarborough, ON, Canada) and Trasylol (Bayer Canada, Etobicoke, ON, Canada). Blood samples for corticosterone were collected in chilled tubes containing heparin. After plasma was removed, packed erythrocytes were resuspended in heparinized saline (10 U.S. pharmacopeia units/mL) containing 1% BSA and reinfused into the rat. After blood samples were obtained at $t$ = 0, an intravenous insulin bolus (10 units/kg) was administered. For achievement of hypoglycemia with as little insulin administered as possible, an intravenous insulin infusion (50 mU/kg/min) was commenced and terminated at the experimenter's discretion. Infusions were delivered via digital pumps (PHD 22/2000 syringe pumps; Harvard Apparatus, Holliston, MA), and both the volume of insulin infused and time when infusion was stopped were recorded. The purpose of Expt-D1 was to attempt to determine the minimal amount of insulin necessary to induce hypoglycemia (2.0–3.5 mmol/L) without causing coma or convulsions and to serve as the control for glucose levels and hormonal responses for Expt-D2. Determining the insulin dosage specifically for each rat on Expt-D1 was necessary since insulin sensitivities of these diabetic rats varied. It is worth noting that neither glucose infusions nor SSTR2a was given on Expt-D1, since it was important to examine each animal's capacity to counterregulate after recurrent hypoglycemia. On Expt-D2, rats were randomly allocated to SSTR2a ($n$ = 12) or placebo ($n$ = 7) treatment. A greater number of rats were given the SSTR2a, since it was expected that results would be more variable in this group. The insulin regimen on Expt-D2 was identical to that used on Expt-D1 for a given rat so that any differences in treatment (placebo vs. SSTR2a) could be observed. Infusion of SSTR2a (PRL-2903, 3000 nmol/kg/min at 1 mL/h) was commenced at $t$ = −60 min and continued for 5-h duration of the experiment, as previously described (26), to determine the effect of SSTR2a on the depth and duration of hypoglycemia. In the placebo-treated group, saline was infused in place of SSTR2a. At the end of 240 min on Expt-D2, all rats were quickly killed by decapitation.

**Plasma hormone measurements.** Plasma glucagon and insulin (LINCO Research, St. Charles, MO), catecholamines (LDN, Nordhorn, Germany), and corticosterone (MP Biomedicals, Solon, OH) were measured by radioimmunoassay using commercially purchased kits as previously described (26).

**SSTR2a.** This peptide antagonist (PRL-2903 and BIM-23458) was synthesized and provided by Dr. D. Coy (Tulane University, New Orleans, LA). Solutions of the peptide antagonist dissolved in 1% acetic acid and diluted with 0.9% saline were freshly prepared the morning of the experiment.

**Data analysis.** All data are represented as means ± SEM. Main outcomes were the repeated-measure comparisons of counterregulatory hormones and glycemic responses between Expt-D1 and Expt-D2 in the two groups of rats (placebo vs. SSTR2a treated). Areas under the curve (AUCs) were calculated using Prism software (GraphPad Software, San Diego, CA). Statistical analysis was performed using Statistica software (Statsoft, Tulsa, OK) on the glycemic responses and the AUCs for counterregulatory hormone responses. Glucose measurements taken over time were compared using repeated-measures ANOVA, followed by Duncan post hoc test. Other comparisons between Expt-D1 and Expt-D2 within the same group were assessed using a paired $t$ test, while comparisons between groups were conducted via a two-tailed $t$ test. In all tests, significance was deemed with $P < 0.05$.

## RESULTS

**Daily blood glucose, body weight, and food intake.** In the week prior to recurrent hypoglycemia, fed glucose (23.6 ± 2.1 vs. 25.3 ± 0.9 mmol/L), body mass (372 ± 11 vs. 365 ± 6 g), and food intake (37 ± 2 vs. 39 ± 1 g/day) were similar in rats that would later be divided into the placebo (saline) and SSTR2a-treated groups (all $P > 0.05$, respectively).

**Glycemia during recurrent hypoglycemia treatment.** By design, all rats achieved five similar episodes of recurrent hypoglycemia over 3 days (nadir = 3.0 ± 0.5 mmol/L for an average of 90 min per episode) (Fig. 1*A*).

**Basal blood glucose and plasma hormone levels after recurrent hypoglycemia treatment.** On the mornings of Expt-D1 and Expt-D2, body mass and initial glycemia did not differ between groups (Table 1). Circulating basal (i.e., before treatment and hypoglycemia induction) insulin and counterregulatory hormone levels were also similar between groups (Table 2). Thus, all rats had similar metabolic starting points after recurrent hypoglycemia.

**Plasma insulin and blood glucose levels during Expt-D1 and Expt-D2.** As we endeavored, similar amounts of insulin (bolus and infusion) were administered to both groups on both days (Table 1). Giving the same amount of insulin on both experimental days, within a treatment group, was important so that any changes observed with glycemia would not be attributed to the amount of insulin administered. Peak circulating insulin levels were also similar between groups and between days (placebo group, Expt-D1 89.3 ± 32.9 ng/mL and Expt-D2 71.9 ± 18.7 ng/mL; SSTR2a group, Expt-D1 96.7 ± 15.9 ng/mL and Expt-D2 75.7 ± 12.3 ng/mL; not significantly different).

In the controls, which received saline infusion on both days, the depth (nadir = 2.6 ± 0.4 vs. 2.7 ± 0.3 mmol/L) and duration of hypoglycemia were similar between Expt-D1 and Expt-D2 (Fig. 1*B*). In contrast, in the SSTR2a group, insulin infusion induced a similar rate of glycemic decline to ~4.0 mmol/L by 90 min on both days, but then blood glucose levels diverged as the threshold for hypoglycemia was approached (Fig. 1*C*). More specifically, with SSTR2 antagonism on Expt-D2, both the depth (nadir: 2.9 ± 0.1 vs. 3.7 ± 0.3 mmol/L on Expt-D1 vs. Expt-D2, respectively; $P < 0.01$) and the duration (126 ± 9 vs. 73 ± 13 min on Expt-D1 vs. Expt-D2, respectively; $P < 0.01$) of hypoglycemia were significantly less compared with Expt-D1 (Fig. 1*C*). In addition, with SSTR2a treatment, rats remained in the euglycemic range in recovery and did not develop rebound hyperglycemia, at least for the experimental period examined (up to

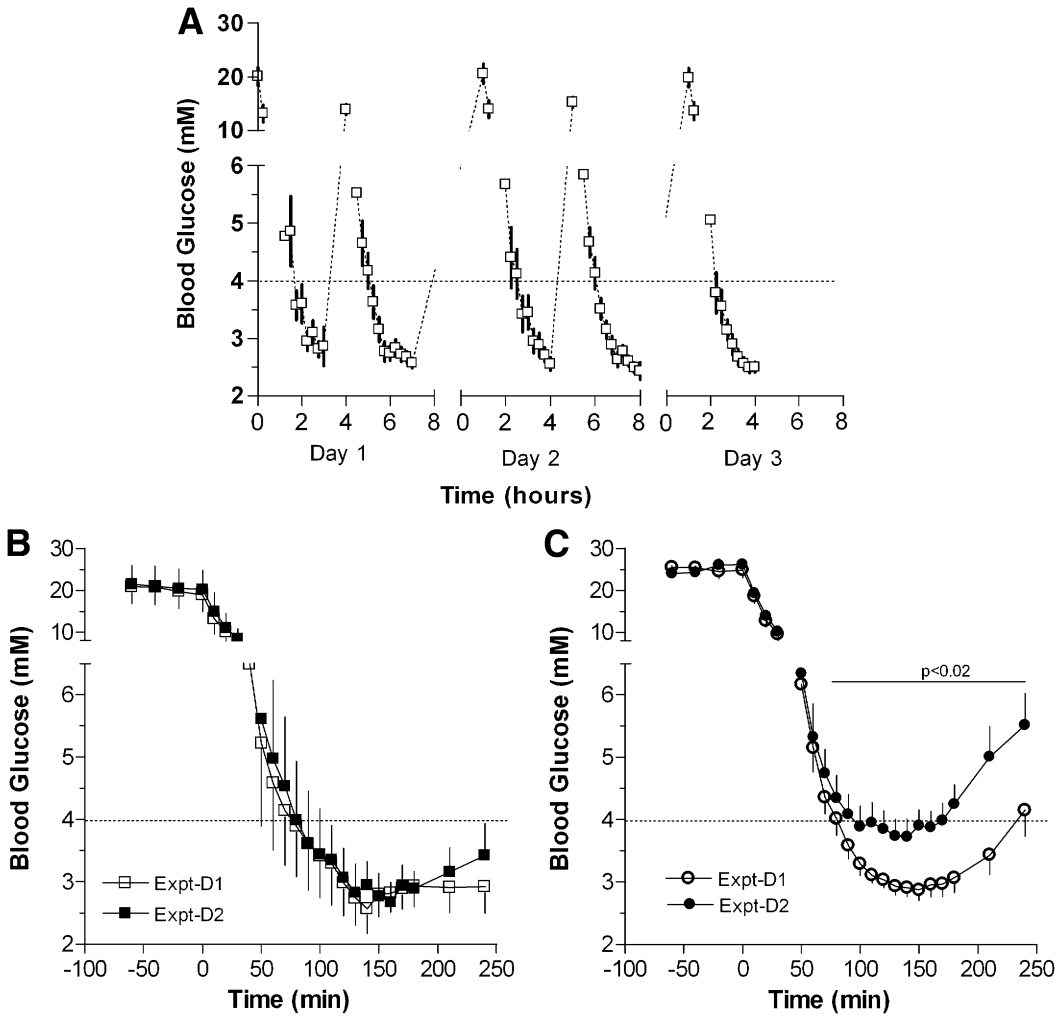


**FIG. 1. Blood glucose responses to repeated insulin-induced hypoglycemia. Antecedent hypoglycemia was induced by subcutaneous insulin injection (10 units/kg) and variable rate glucose infusion over a 3-day conditioning period in all rats ($n$ = 19) (*A*). The next day (Expt-D1), rats were randomly allocated to either the control group ($n$ = 7) (*B*) or the SSTR2a treatment group ($n$ = 12) (*C*) for baseline assessment of counterregulatory responses by using a combination of insulin bolus (10 units/kg i.v.) and infusion (50 mU/kg/min i.v.) at the discretion of the investigator until moderate hypoglycemia ensued (target 3.0 mmol/L) (*B*). One day later, on Expt-D2, the insulin infusion treatment protocol was duplicated for each rat, either with (SSTR2a group) or without (controls) SSTR2a infusion (3,000 nmol/kg/min i.v.), which commenced 60 min prior to insulin treatment. Values are means ± SEM.**

TABLE 1
Body weight and the amount of insulin administered via intravenous bolus and infusion and total insulin administered in both groups on both days

| | Control group (saline-saline) ($n$ = 7) | | Treatment group (saline-SSTR2a) ($n$ = 12) | |
|---|---|---|---|---|
| | Expt-D1 | Expt-D2 | Expt-D1 | Expt-D2 |
| Body mass (g) | 340 ± 10 | 338 ± 10 | 341 ± 11 | 340 ± 10 |
| Baseline glycemia (mmol/L) | 20.9 ± 4.0 | 21.5 ± 4.4 | 25.5 ± 1.7 | 24.1 ± 1.9 |
| Insulin via intravenous bolus (units) | 3.12 ± 0.63 | 3.09 ± 0.64 | 3.24 ± 0.27 | 3.26 ± 0.29 |
| Insulin via intravenous infusion (units) | 0.89 ± 0.30 | 0.89 ± 0.30 | 0.88 ± 0.11 | 0.90 ± 0.11 |
| Total insulin administered (units) | 4.01 ± 0.86 | 3.98 ± 0.85 | 4.12 ± 0.31 | 4.16 ± 0.34 |

Data are means ± SEM. On Expt-D1, rats received 10 units/kg i.v. bolus of insulin after basal samples were obtained at time = 0 min. Subsequently, insulin infusion (50 mU/kg/min) was started and stopped at the experimenter's discretion when the rat's blood glucose approached hypoglycemia. The volume, timing, and rate of insulin infusion were recorded and repeated for each individual rat on Expt-D2. No difference existed either between groups or between experimental days.

240 min post–insulin administration). The extent of hypoglycemia, calculated as the AUC <4.0 mmol/L, was considerably less with SSTR2a treatment compared with saline treatment (10 vs. 90 mmol/L/min, $P < 0.001$). Since some rats still developed hypoglycemia with SSTR2a treatment, albeit in a milder form, the percent of animals with blood glucose levels <4.0 and <3.5 mmol/L were also plotted for Expt-D2 only, since this was the only day in which the treatments differed (Fig. 2*A* and *B*). In this analysis of drug efficacy, the percentage of rats with blood glucose levels <4.0 and <3.5 mmol/L were higher in the rats given SSTR2a (~33 and 40%, respectively) compared with rats given placebo (~0 and 8%, respectively).

**Counterregulatory hormone levels during Expt-D1 and Expt-D2.** Since baseline levels of all counterregulatory hormones differed slightly between groups and between days (Table 2), their responses to hypoglycemic treatment were plotted for both groups (Fig. 3*A*–*F*). After 3 days of recurrent hypoglycemia, glucagon responses to hypoglycemia on Expt-D1 were modest in both groups (Fig. 3*A* and *B*). On Expt-D2, the glucagon response to hypoglycemia in controls diminished markedly (AUC decreased by >20-fold, $P < 0.05$), while it improved significantly in the SSTR2a-treated group (AUC increased threefold, $P < 0.05$).

In controls, the corticosterone responses to hypoglycemia were relatively robust on Expt-D1 but diminished by approximately one-half on Expt-D2 ($P < 0.05$) (Fig. 3*C*). In contrast, the corticosterone response to hypoglycemia in the SSTR2a group on Expt-D1 was somewhat attenuated compared with controls but tended to improve with SSTR2a treatment on Expt-D2 ($P = 0.2$ for AUC analysis) (Fig. 3*D*).

Epinephrine levels increased in the controls on Expt-D1, but responses were significantly attenuated on Expt-D2 ($P < 0.05$ for AUC) (Fig. 3*E*). Interestingly, catecholamine responses were less robust in the SSTR2a group on both experimental days compared with controls and were still attenuated on Expt-D2 compared with Expt-D1 (Fig. 3*F*). Since the apparent attenuation in epinephrine response on Expt-D2 compared with Expt-D1 despite SSTR2a treatment may have been related to improved counterregulation in other hormones and to higher glycemic values overall, we also compared the catecholamine responses between Expt-D1 and -2 in the six rats who still developed hypoglycemia with SSTR2a treatment. In this analysis, we observed that SSTR2a was associated with a preserved epinephrine response to recurrent hypoglycemia (data not shown). Norepinephrine response to hypoglycemia was similar both between groups and between experimental days (data not shown).

## DISCUSSION

This study is the first to show that the use of a selective SSTR2 inhibitor reduces the likelihood of insulin-induced hypoglycemia in diabetic rats that have developed counterregulatory failure because of repeated exposure to recurrent hypoglycemia. This novel finding may have significant implications for the development of new prophylactic therapies targeting SSTR2 inhibition for hypoglycemia prevention in type 1 diabetes mellitus.

Our prior work has demonstrated that SSTR2 antagonism improves some of the counterregulatory hormone response to hypoglycemic clamp in diabetic rodents naive to prior hypoglycemia (26). This study extends these findings by

TABLE 2
Basal plasma hormone levels on the morning of Expt-D1 and -D2 in rats that would subsequently undergo hypoglycemia treatment

| | Control group (saline-saline) ($n$ = 7) | | Treatment group (saline-SSTR2a) ($n$ = 12) | |
|---|---|---|---|---|
| | Expt-D1 | Expt-D2 | Expt-D1 | Expt-D2 |
| Insulin (ng/mL) | 1.00 ± 0.2 | 0.97 ± 0.2 | 0.98 ± 0.2 | 0.98 ± 0.1 |
| Glucagon (pg/mL) | 55 ± 5 | 53 ± 5 | 58 ± 5 | 60 ± 6 |
| Epinephrine (pg/mL) | 126 ± 33 | 129 ± 29 | 106 ± 17 | 102 ± 21 |
| Norepinephrine (pg/mL) | 401 ± 52 | 378 ± 72 | 326 ± 77 | 300 ± 77 |
| Corticosterone (ng/mL) | 110 ± 37 | 132 ± 28 | 76 ± 20 | 59 ± 14 |

Data are means ± SEM. No significant differences existed either between groups or between days.

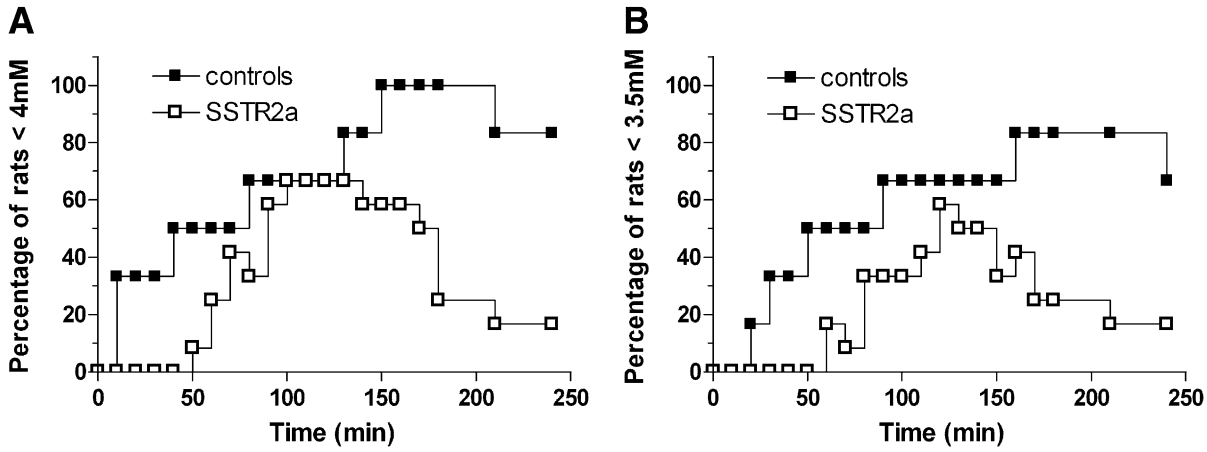


**FIG. 2. Percentage of rats that developed hypoglycemia, as measured by a blood glucose <4.0 mmol/L (*A*) or <3.5 mmol/L (*B*) in the control and SSTR2a-treated groups on Expt-D2.**

showing that rodents exposed to recurrent hypoglycemia have improvements in their glucagon and corticosterone, but not catecholamine, responses to subsequent hypoglycemia when an SSTR2a is administered. More importantly, animals are shown to be more resistant to insulin-induced hypoglycemia with SSTR2a treatment. These findings are particularly relevant, since reducing the hypoglycemic nadir and reducing the duration of hypoglycemic exposure are both important in preserving normal brain function and preventing severe neuroglucopenia, seizures, and loss of consciousness or death (3). If selective SSTR2 antagonism is demonstrated to promote hypoglycemic resistance in the long term, without adverse side effects, then insulin therapies that include SSTR2 antagonism may have wider latitude for safety patients living with type 1 diabetes mellitus.

Taking together the results of our prior study using SSTR2 inhibition (26) and the observations that there are elevations in pancreatic gene expression and somatostatin levels in diabetes mellitus (16–20), we propose here that increased somatostatin concentration and/or signaling is one of the key contributing factors in the development of glucose counterregulation failure in type 1 diabetes mellitus. However, this study also reveals that some rodents still develop hypoglycemia even when somatostatin inhibition exists (~50% [Fig. 2]), perhaps because the glucagon response to hypoglycemia is not fully restored in rats exposed to recurrent hypoglycemia. Indeed, a comparison of glucagon responses in this study with that of nondiabetic rodents and rodents given SSTR2a during their first bout of hypoglycemia in our prior work (26) suggests that the decrement in counterregulatory responses to recurrent hypoglycemia is not fully restored by SSTR2 antagonism (~30 vs. 225 pg/mL glucagon response in this study compared with our prior study). Nonetheless, the ability of the SSTR2a to help preserve blood glucose levels >3.5 mmol/L in rats previously exposed to frequent hypoglycemia may have clinical relevance, as an earlier study demonstrated that hypoglycemia at 3.3 mmol/L reduced cognitive function (30). It remains to be determined, however, whether prolonged SSTR2 antagonism therapy can limit the frequency of insulin-induced hypoglycemia in type 1 diabetes mellitus or whether it has any efficacy in limiting the high rate of occurrence of hypoglycemia in type 2 diabetes mellitus.

Hypoglycemia, even when symptom free, leads to defective glucose counterregulation and hypoglycemia unawareness (31,32). Since these episodes substantially increase the risk of subsequent hypoglycemia, all hypoglycemic events place the individual at elevated risk for future (and more catastrophic) occurrences (1). It has been suggested that delayed recovery of hypoglycemia may frequently occur in type 1 diabetic individuals in whom deficient epinephrine and glucagon counterregulation result in impaired hepatic glucose release (33). In patients with type 1 diabetes mellitus, avoidance of hypoglycemia helps to restore hypoglycemia awareness and perhaps glucose counterregulation (5,34–36). This avoidance is typically achieved by a relaxation in insulin therapy, which can cause a deterioration in glycemic control, as measured by A1C levels (34–36). Whether SSTR2a treatment deteriorates insulin sensitivity or allows for a restoration in glucose counterregulation and hypoglycemia avoidance without any alterations in insulin therapy remains to be established. In this study, it would appear that the insulin pharmacokinetics, as assessed by the rate of change in glucose after intravenous insulin administration, were unchanged with SSTR2a treatment, which may be considered beneficial for overall patient control (Fig. 1*C*).

In our prior study, we observed that SSTR2a appeared to promote an increase in glucagon release only during hypoglycemia (26). In this study, it would appear that the SSTR2a may increase glucagon release well before hypoglycemia ensues. Indeed, the glucagon response in the SSTR2a-treated animals rose well before the onset of hypoglycemia (Figs. 1 and 3). This suggests that the antagonist may trigger enhanced hormone release in response to a decline in glycemia rather than to hypoglycemia per se. Although our prior work suggests that the provision of the same SSTR2a does not influence insulin sensitivity or glucose production during euglycemia or hyperglycemia (26), a more generic effect of SSTR2a treatment on glucagon release cannot be ruled out at this time.

There is little doubt that somatostatin levels are increased with diabetes. Prosomatostatin mRNA expression in diabetic rats is elevated in islets compared with nondiabetic rats, and these levels remain elevated even after seven episodes of recurrent hypoglycemia (7). In humans with type 1 diabetes mellitus, circulating somatostatin

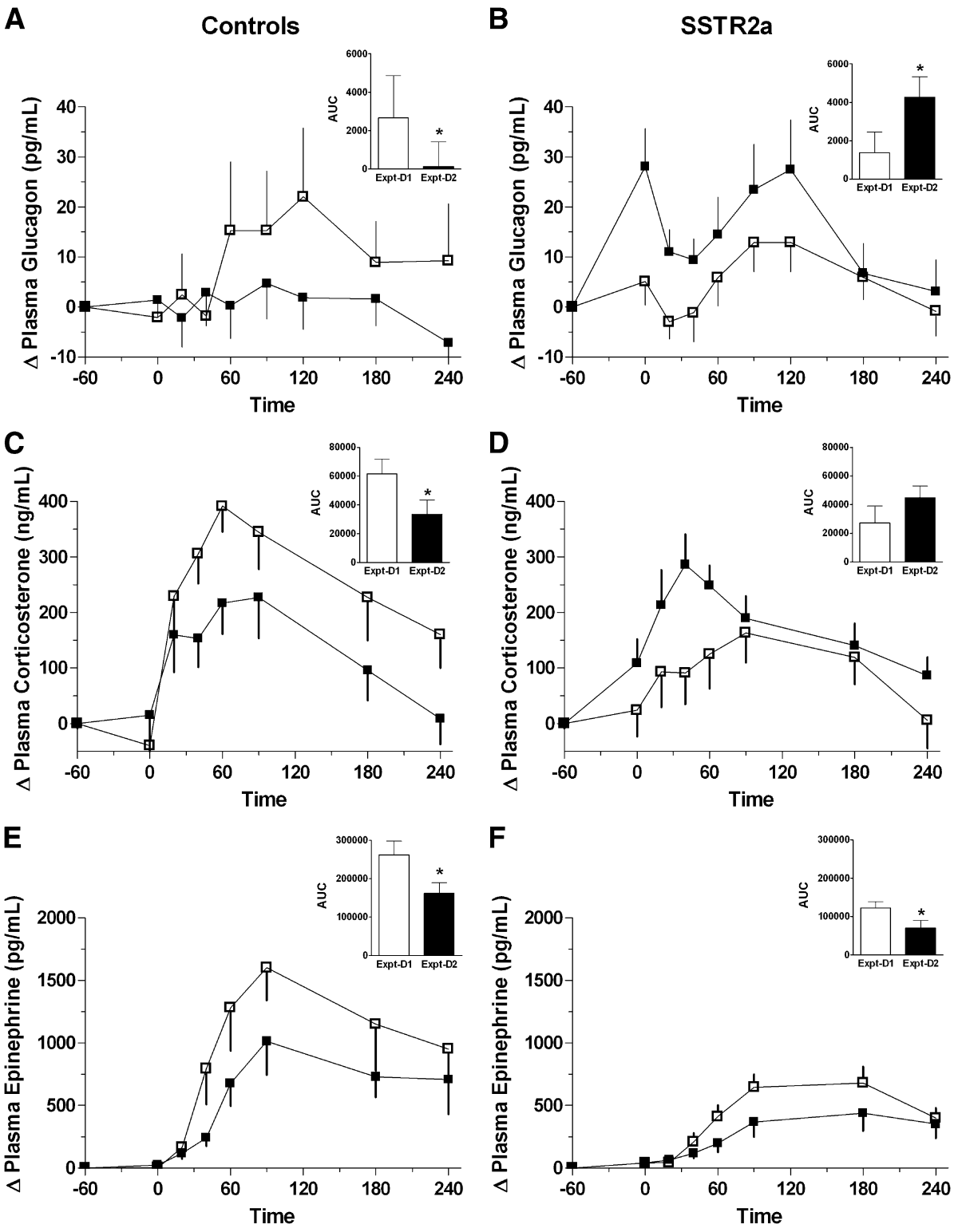


**FIG. 3. Counterregulatory hormone responses to hypoglycemia in control and SSTR2a-treated rats on Expt-D1 and -D2. Plasma glucagon responses (*A* and *B*), plasma corticosterone responses (*C* and *D*), and plasma epinephrine responses (*E* and *F*) were determined for Expt-D1 (□) and Expt-D2 (■) in both groups. The integrated AUC for the hormonal responses to hypoglycemia is also shown (*insets*). Values are means ± SEM. *Expt-D2 was significantly different from Expt-D1 at $P < 0.05$.**

levels are elevated (16,17,19) and pancreatic somatostatin levels are elevated by >10-fold (37,38), particularly in those with poor glycemic control (38). Thus, as with acute hypoglycemia, we propose here that increased pancreatic somatostatin levels may play a role in impairing glucagon release after recurrent hypoglycemia. Using this same selective SSTR2a, stimulated secretion of glucagon, but not insulin, is dose-dependently enhanced in perifused islets and in perfused pancreata of healthy rats (27). The same antagonist also reverses the suppressive effects of an

SSTR2 agonist on arginine-stimulated glucagon secretion in isolated human islets (39). Based on the improved glucagon responses to hypoglycemia that we previously observed with SSTR2a treatment (26) and on our observations in this study (Fig. 3*B*), we assume that the effectiveness of SSTR2 antagonism on hypoglycemia prevention after recurrent hypoglycemia is primarily related to enhanced glucagon-mediated hepatic glycogenolysis. However, other possible mechanisms do exist. It may be that somatostatin antagonism increases glucose production or lowers glucose disposal. At present, however, there is no known direct effect of SSTR2 antagonism on hepatic glycogenolysis or gluconeogenesis. Prolonged infusion of SSTR2a under basal, nonclamped conditions does not appear to affect glucose turnover (26). Moreover, SSTR1 and SSTR3, but not SSTR2, have been detected on hepatocytes (40,41) and the SSTR2a used in this study has no reactivity with SSTR1 and 10-fold less binding affinity with SSTR3 (42). As previously observed (26), corticosterone levels tend to be increased during hypoglycemia when SSTR2a is given (Fig. 3*D*). Elevations in corticosterone would also be expected to enhance hepatic glucose production and possibly limit peripheral glucose uptake. Interestingly, a direct effect of somatostatin to enhance insulin-stimulated muscle glucose uptake, but not basal muscle glucose uptake, has been demonstrated in humans (43). Thus, it is plausible that inhibition of somatostatin in the current study could contribute to reduced muscle glucose clearance during hypoglycemia, although overall insulin sensitivity did not appear to be impacted by the use of the antagonist in this study. Evidence of SSTR subtypes on skeletal muscle is scarce, but SSTR2, SSTR3, and SSTR4 mRNA has been detected in rat skeletal muscle (44). Thus, a global reduction in somatostatin signaling may promote several antihypoglycemic mechanisms, some of which remain to be identified.

In addition to attenuated glucagon and corticosterone response to hypoglycemia, the catecholamine responses are also lost (7,8). Somatostatin is thought to inhibit epinephrine release by receptor-coupled signaling initiated by acetylcholine-nicotinic receptor binding, subsequent membrane depolarization, and intracellular calcium increase (45), resulting in the consequent inhibition of adrenomedullary epinephrine secretion from the adrenal medulla. However, in our studies, SSTR2a did not improve the catecholamine response to hypoglycemia (Fig. 3*F*). We speculate that the apparent attenuation in epinephrine response to hypoglycemia was because glycemia did not reach the same nadir when SSTR2a was used, likely because the glucagon response was improved. Indeed, in the six rats that developed hypoglycemia despite SSTR2a treatment, the epinephrine response was identical to that observed on Expt-D1. Thus, we conclude that SSTR2a treatment does not significantly alter the catecholamine response to hypoglycemia, at least in rats that had diabetes for a relatively short period of time.

Our study has a number of limitations that should be mentioned. First, in spite of identical hypoglycemic conditioning leading up to the experimental days, the two groups of rats examined had different glucose counterregulatory responses to the hypoglycemia on Expt-D1. Indeed, the corticosterone and epinephrine responses in the rats that would receive SSTR2a the next day appeared to be slightly more impaired compared with the placebo group (Fig. 3), which may have exaggerated the effectiveness of the antagonist on Expt-D2. On the other hand, if this group of rats did have significantly attenuated counterregulatory responses, one may consider that the antagonist is indeed effective even in animals that have completely abolished glucose counterregulation. Second, because of limitations in blood collection we did not measure the growth hormone response to hypoglycemia in this study, which may have been improved with SSTR2a treatment. In our previous publication (26), we did not observe an effect of SSTR2a inhibition on growth hormone release during hypoglycemia (unpublished data). However, given that growth hormone is triggered by hypoglycemia (45) and suppressed by somatostatin primarily via the type 2 receptor (46,47), further studies are needed to determine whether SSTR2a treatment also helps to augment growth hormone release during hypoglycemia.

In conclusion, we demonstrate that hypoglycemia can be ameliorated by SSTR2 antagonism after recurrent hypoglycemia in diabetic rats, presumably at least in part by enhancing glucagon and corticosterone counterregulation. These results also help to support the role for pancreatic, and possibly circulating, somatostatin in attenuating the counterregulatory response in diabetes and that this defect can be countered using a pharmacological dose of SSTR2a. Although further investigation is necessary to elucidate the exact mechanisms by which euglycemia is restored by inhibiting somatostatin action and whether any other deleterious off-target effects occur because of regular treatment, these findings hold promise for a new pharmacotherapy for hypoglycemia prevention in type 1 diabetes mellitus.

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J.T.Y.Y. researched data and wrote the manuscript. M.C.R. assisted with the research data analysis and interpretation and the writing of the manuscript. E.B. researched data. D.H.C. provided the antagonist, assisted with the research design, and edited the manuscript. S.E. contributed to discussion and reviewed and edited the manuscript. M.V. oversaw the research design and interpretation of data and reviewed and edited the manuscript. M.C.R. is the guarantor of this work and as such had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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