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The selective serotonin reuptake inhibitor sertraline enhances counterregulatory responses to hypoglycemia

Nicole M. Sanders[1,2], **Charles W. Wilkinson**[2,3], **Gerald J. Taborsky Jr.**[1,4], **Salwa Al-Noori**[2,5], **Wendi Daumen**[5], **Aryana Zavosh**[5], and **Dianne P. Figlewicz**[1,2]

1*Divisions of Endocrinology/Metabolism, Veterans Affairs Puget Sound Health Care System*

2*Departments of Psychiatry and Behavioral Sciences, University of Washington*

3*Geriatric Research, Education and Clinical Center, Veterans Affairs Puget Sound Health Care System*

4*Department of Medicine, University of Washington*

5*Seattle Institute for Biomedical and Clinical Research, Seattle, Washington*

Abstract

Selective serotonin reuptake inhibitors (SSRIs) are widely prescribed for patients with comorbid diabetes and depression. Clinical case studies in diabetic patients, however, suggest that SSRI therapy may exacerbate hypoglycemia. We hypothesized that SSRIs might increase the risk of hypoglycemia by impairing hormonal counterregulatory responses (CRR). We evaluated the effect of the SSRI sertraline on hormonal CRR to single or recurrent hypoglycemia in nondiabetic rats. Since there are time-dependent effects of SSRIs on serotonin neurotransmission that correspond with therapeutic action, we evaluated the effect of 6- or 20-day sertraline treatment on hypoglycemia CRR. We found that 6-day sertraline (SERT) treatment specifically enhanced the epinephrine response to a single bout of hypoglycemia vs. vehicle (VEH)-treated rats (t = 120: VEH, 2,573 ± 448 vs. SERT, 4,202 ± 545 pg/ml, $P < 0.05$). In response to recurrent hypoglycemia, VEH-treated rats exhibited the expected impairment in epinephrine secretion (t = 60: 678 ± 73 pg/ml) vs. VEH-treated rats experiencing first-time hypoglycemia (t = 60: 2,081 ± 436 pg/ml, $P < 0.01$). SERT treatment prevented the impaired epinephrine response in recurrent hypoglycemic rats (t = 60: 1,794 ± 276 pgl/ml). In 20-day SERT-treated rats, epinephrine, norepinephrine, and glucagon CRR were all significantly elevated above VEH-treated controls in response to hypoglycemia. Similarly to 6-day SERT treatment, 20-day SERT treatment rescued the impaired epinephrine response in recurrent hypoglycemic rats. Our data demonstrate that neither 6- nor 20-day sertraline treatment impaired hormonal CRR to hypoglycemia in nondiabetic rats. Instead, sertraline treatment resulted in an enhancement of hypoglycemia CRR and prevented the impaired adrenomedullary response normally observed in recurrent hypoglycemic rats.

Keywords

epinephrine; adrenomedullary; hypoglycemia-associated autonomic failure

INDIVIDUALS WITH DIABETES exhibit a twofold higher rate of depression compared with the general population (29). Comorbid depression and diabetes are associated with hyperglycemia and poor glycemic control (25), an accelerated progression of complications

Address for reprint requests and other correspondence: N. M. Sanders, VA Puget Sound Health Care System, Metabolism and Endocrinology (S-151), 1660 So. Columbian Way, Seattle, WA 98108 (e-mail: sandersn@u.washington.edu)..

associated with diabetes (8,26), and an increased risk of mortality (41). Selective serotonin reuptake inhibitors (SSRIs) are the drug of choice for the treatment of depression. The majority of clinical studies support the use of SSRIs in comorbid diabetes and depression (16,26,27). Diabetic patients on SSRI therapy can exhibit reduced fasting glucose levels, reduced body weight, improved glycemic control, and improved hemoglobin A_{1c} values compared with diabetic patients on other commonly prescribed antidepressants (15,28). Furthermore, SSRI therapy effectively reduces depression recurrence in diabetic patients (27).

Similarly to other antidepressant therapies (16), SSRIs can impact blood glucose levels; thus they can present potential risks to individuals with diabetes. Of particular significance, SSRI therapy in diabetic individuals has been associated with an increase in the frequency and severity of hypoglycemia and absence of hypoglycemia symptoms (10,43,49). Hypoglycemia associated with SSRI therapy may be particularly problematic for intensively managed type 1 and type 2 diabetic patients. Intensive insulin, or glucose lowering, therapies aimed at maintaining tight glycemic control are associated with a three- to fourfold increase in the incidence of severe hypoglycemia (1). As a result of recurrent antecedent hypoglycemia, the hormonal counterregulatory responses (CRRs) normally elicited by decreases of plasma glucose become impaired, thus increasing the risk for future bouts of hypoglycemia (9). Impaired hypoglycemia counterregulation is a component of the clinical syndrome of hypoglycemia-associated autonomic failure (HAAF). Thus, the use of SSRI therapy in the background of diabetes and intensive glucose-lowering therapy might further exacerbate the risk of severe hypoglycemia.

Serotonergic mechanisms have long been known to modulate neuroendocrine responses that are also critical to hypoglycemia counterregulation. Thus, the association between SSRIs and hypoglycemia may be due to SSRI-induced impairment(s) of central mechanisms mediating hypoglycemia-induced CRRs. In line with this idea, acute treatment with the SSRI sertraline in humans suppresses basal sympathetic nervous system activity (45). In addition, it has been hypothesized that serotonin neurons located in the caudal hindbrain are directly sensitive to changes in glucose availability (i.e., glucose sensing) (30). Therefore, SSRIs may reduce the sensitivity of this particular population of glucose-sensing neurons that contributes to the activation of hormonal CRRs.

In the present study, we employed our model of single vs. recurrent bouts of hypoglycemia to directly identify the effects of sertraline treatment on autonomic, neuroendocrine, and behavioral (stimulation of food intake) CRRs to hypoglycemia. Our 2-day recurrent hypoglycemia model induces significant impairments in hormonal CRRs to a subsequent bout of hypoglycemia (i.e., animal model of HAAF) (14). Given the incidence of inadvertent hypoglycemia among patients on SSRI therapy reported in the clinical literature (10,43,49), we hypothesized that sertraline treatment would impair hormonal CRRs to a single bout of hypoglycemia and further impair CRRs in our model of HAAF. Surprisingly, we found that continuous sertraline delivery for either 6 or 20 days enhanced, rather than impaired, key hypoglycemia CRRs epinephrine, glucagon, and norepinephrine. Perhaps more importantly, we report that both sertraline treatment regimens (6 or 20 days) corrected the impaired adrenomedullary response observed in our rodent model of HAAF. Thus, it is not likely that the clinical findings of increased incidence of hypoglycemia with SSRI therapy are the result of an impaired hormonal CRR, since our findings in normal nondiabetic rats clearly demonstrate an enhancement of key hormonal responses to hypoglycemia, even under conditions of recurrent hypoglycemia.

MATERIALS AND METHODS

Subjects

Adult male Sprague-Dawley rats purchased from Simonsen Laboratories (Gilroy, CA) were used for these experiments. Rats were housed individually and maintained on a 12:12-h light-dark schedule (lights on at 6 AM, lights off at 6 PM) at 22−23°C with ad libitum access to food (Purina Rat Chow, no. 5001) and water, except where specified otherwise. All procedures were approved by the Animal Studies Subcommittee of the Veterans Affairs (VA) Puget Sound Health Care System Research and Development Committee, Seattle Division.

Surgery

All rats were surgically implanted with a Silastic intravenous (iv) catheter into the jugular and submaxillary vein under ketamine-xylazine [86 mg/kg ketamine (KetaFlo; Abbott Laboratories, Chicago, IL), 12.9 mg/kg xylazine (Xyla-ject; Phoenix Pharmaceutical, St. Josesph, MO)] anesthesia as described previously (14). The catheters were tunneled subcutaneously and exteriorized through a midline incision in the scalp. The catheters were held in place with four skull screws (Small Parts, Miami Lakes, FL) and acrylic cement (Lang Dental, Wheeling, IL). Rats received 3 ml sc of lactated Ringer solution (Baxter Pharmaceutical Products, New Providence, NJ) and buprenorphine analgesia (0.05 mg/kg sc) and were maintained on a circulating water heating pad until recovery from anesthesia. Catheter lines were filled with 60 or 20% polyvinylpyrrolidone (PVP10; Sigma, St. Louis, MO)/heparin (1,000 U/ml; Elkins-Sinn, Cherry Hill, NJ) and kept patent by a heparin (10 U/ml) flush every 3 days.

Sertraline treatment

Rats were treated with the SSRI sertraline (Toronto Research Chemicals), 7.5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 6 or 20 days subcutaneously, by means of osmotic minipumps (Model 2001 or 2ML4; Alza, Palo Alto, CA). The concentration, duration, and means of sertraline delivery have previously been shown to produce stable sertraline serum concentrations similar to those recommended therapeutically (5). Control groups received vehicle (50% ethanol-distilled water). Minipumps were inserted under isoflurane anesthesia (~10 min procedure) after rats had recovered from catheterization surgery (regained weight to at least the presurgical level, ~7 days). A 1- to 1.5-cm incision was made between the scapulae and the pump inserted subcutaneously. The incision was closed with two skin staples.

Experimental procedures

The 6- and 20-day studies were carried out separately. The experimental procedures for each study were identical, other than the duration of drug or vehicle treatment. Six- and 20-day sertraline (SERT-) and vehicle (VEH)-treated rats were subjected to our 2-day (*days 1* and *2*) testing procedure (14) that coincided with the last 2 days of SERT or VEH treatment (*days 5* and *6* or *days 19* and *20*). All testing procedures were conducted in square acrylic testing chambers (~30 × 30 × 30 cm) to allow for remote blood collection. Prior to the experiment being initiated, rats were familiarized with the testing chambers (4 h/day for 4 days). SERT- and VEH-treated rats were subjected to the following experimental treatments: saline controls (SAL; 2 saline infusions on *day 1* and a single saline infusion on *day 2*), single hypoglycemia (SH; 2 saline infusions on *day 1* and insulin-induced hypoglycemia on *day 2*), and recurrent hypoglycemia (RH; 2 insulin infusions on *day 1* and 1 insulin infusion on *day 2*). Hypoglycemia was induced by an iv infusion (1.146 ml/h, microinfusion pump; KD Scientific) of insulin ($0.125\ U \cdot 100\ g^{-1} \cdot 2\ h^{-1}$, Novolin R, regular humulin insulin, recombinant DNA origin; Novo Nordisk, Princeton, NJ). Control rats received an iv infusion of sterile saline.

On the first day of testing (*day 1*), SERT- and VEH-treated rats were infused (iv) with either insulin to induce hypoglycemia (one or two 2-h bouts of hypoglycemia separated by a 60-min interval) or saline vehicle. Food was available during the 60-min interval. Blood (0.1 ml) was drawn immediately before the onset of insulin or saline infusion and 60 and 120 min thereafter for measurement of plasma glucose to confirm hypoglycemia or euglycemia, respectively. On *day 2* of testing, rats were infused with insulin or saline. Blood was collected (1.5 ml) immediately prior to insulin or saline infusion ($t = 0$) and 60 and 120 min thereafter for subsequent measurement of plasma glucose, glucagon, epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), and corticosterone. Blood was immediately replaced with donor blood drawn from unstressed rats prior to the experiment. At the completion of the 2-h insulin or saline infusion, preweighed rat chow was returned to the testing chambers and 2-h food intake measured.

Plasma assays

Blood samples were obtained for the measurement of hormonal responses and stored at −80° C until assayed. Blood for the catecholamine assays was collected on EGTA-glutathione (2.3:1.5 mg/ml; Sigma). Tubes for glucagon assays contained 50 μl of 1 M benzamidine (Sigma) and 1 U heparin. Blood for glucose, ACTH, and corticosterone assays was collected on EDTA and aprotinin (1.7 tissue inhibitor unit; Sigma). The assays have been described previously (14). Briefly, a radioenzymatic method described by Evans et al. (13) was used for determination of plasma epinephrine and norepinephrine. An RIA procedure was used for plasma corticosterone measurement (38). Plasma glucose was measured using the Beckman glucose analyzer. Glucagon was assayed by the Linco glucagon RIA kit (Linco Research, St. Charles, MO). Plasma ACTH concentration was measured with an immunoradiometric assay kit (Scantibodies Laboratory, Santee, CA). The assay was performed according to the commercial protocol, with the exception of the use of 150 μl of sample volume instead of 200 μl. Sensitivity of the assay is 2 pg/ml, and intra- and interassay coefficients of variation were 4.2 and 7.6%, respectively. Assay performance has been characterized completely for measurement of human ACTH (50).

Statistical analysis

The data collected from the 6- and 20-day studies were analyzed separately. Data from the plasma assays were analyzed using two-factor repeated-measures ANOVA [time × hypoglycemia (SAL, SH, or RH) or time × treatment (VEH or SERT)] for overall effects. Specific post hoc comparisons were carried out using Student's *t*-test. Significance for all tests was taken as $P \leq 0.05$. Feeding data were analyzed by ANOVA.

RESULTS

Six-day sertraline study

Body weight data for VEH- and SERT-treated rats are presented in Table 1. Although SERT-treated rats tended to weigh less in each experimental treatment group (SAL, SH, and RH), this did not reach statistical significance. Glucose and neuroendocrine data for the experimental groups in the 6-day study are provided in Table 2. Baseline ($t = 0$) plasma glucose and hormone levels were matched between VEH- and SERT-treated rats in each of the experimental groups. There was no effect of 6-day SERT treatment on glucose or hormones during the SAL control infusion; rather, the consequence of SERT treatment was observed only in response to insulin-induced hypoglycemia. Six-day SERT treatment significantly increased the epinephrine response to a single bout of hypoglycemia [$F(1,21) = 4.1$, $P = 0.05$; Table 2 and Fig. 1]. Post hoc analysis revealed that epinephrine levels were significantly increased during SH in SERT- vs. VEH-treated rats at $t = 120$ ($P = 0.032$). In contrast, SERT treatment did not significantly affect glucose [$F(1,18) = 0.221$, $P = 0.644$], glucagon [$F(1,19) = 1.62$, $P = 0.219$],

norepinephrine [$F(1,21) = 0.226$, $P = 0.639$], ACTH [$F(1,21) = 1.737$, $P = 0.202$], or corticosterone [$F(1,21) = 1.963$, $P = 0.176$] responses to SH compared with VEH-treated rats (Table 2 and Fig. 1). Thus, 6-day SERT treatment specifically enhanced the epinephrine response to a single bout of insulin-induced hypoglycemia.

Similar to our previous studies (14), hypoglycemia-induced hormonal CRRs were blunted in our acute rodent model of HAAF. There was a significant decrease in epinephrine [$F(1,18) = 11.9$, $P = 0.003$; $t = 60$, $P = 0.01$; $t = 120$, $P = 0.01$] and glucagon [$F(1,18) = 9.0$, $P = 0.007$; $t = 60$, $P = 0.01$; $t = 120$, $P = 0.02$] secretion in RH VEH-treated rats during their third bout of hypoglycemia compared with VEH-treated rats experiencing their first bout of hypoglycemia (SH) (Table 2). There was a significant treatment effect of SERT in RH rats to increase epinephrine levels compared with RH VEH-treated rats [$F(1,20) = 7.7$, $P = 0.012$; $t = 60$, $P = 0.004$; $t = 120$, $P = 0.028$]. In fact, SERT treatment essentially rescued the impaired epinephrine response in RH rats because epinephrine levels were closely matched in RH SERT and SH VEH rats [$F(1,22) = 32.80$, $P = 0.67$; Fig. 2]. Similar to our findings in the SH SERT treatment group (see previous paragraph), epinephrine was the only hormonal CRR affected by 6-day SERT treatment in response to RH (Table 2).

Hypoglycemia stimulates a robust increase in food intake (46). This behavioral response to hypoglycemia is important because it ensures the restoration of depleted glycogen stores, which, in turn, can lessen the overall magnitude of hypoglycemia. In the present study, we evaluated the effect of SERT treatment on hypoglycemia-induced food intake (Table 4). Two-hour food intake immediately following the control SAL infusion did not differ between VEH- and SERT-treated rats [$F(1,19) = 2.68$, $P = 0.118$]. Hypoglycemia produced similar increases in food intake in VEH- and SERT-treated rats in response to SH [$F(1,26) = 0.005$, $P = 0.944$] or RH [$F(1,37) = 0.115$, $P = 0.737$] (Table 4). Similar to our previous findings (42), stimulation of food intake, unlike hormonal responses, was not impaired in VEH- or SERT-treated rats subjected to our acute model of recurrent hypoglycemia.

Twenty-day sertraline study

The body weights of VEH- and SERT-treated rats on *day 1* of the 2-day experiment are provided in Table 1. There were no significant differences in body weight between VEH- and SERT-treated rats in each of the experimental groups (SAL, SH, and RH). Plasma glucose and neuroendocrine data for the experimental groups in the 20-day study are shown in Table 3. Baseline ($t = 0$) glucose and hormone levels are matched between VEH and SERT rats in each of the chronic treatment groups. Glucose and hormone levels during the SAL control infusion were similar between VEH-and SERT-treated rats (Table 3). In contrast to the 6-day SERT study, 20-day SERT treatment affected multiple hormonal CRRs to SH and RH. In the SH group, there was an overall treatment effect of SERT on the epinephrine response [$F(1,10) = 11.6$, $P = 0.006$]. Post hoc analysis revealed a significant elevation in epinephrine at both $t = 60$ ($P = 0.01$) and $t = 120$ ($P = 0.05$) in the SERT- vs. VEH-treated group (Table 3). In fact, 20-day SERT treatment was more effective than 6-day SERT treatment in enhancing the epinephrine response to hypoglycemia [$F(1,16) = 6.024$, $P = 0.023$]. Epinephrine levels were significantly elevated in response to SH in 20 vs. 6-day SERT-treated rats at $t = 60$ ($P = 0.037$; Fig. 3). In addition, there was a significant increase in norepinephrine levels in response to SH in SERT- vs. VEH-treated rats [$F(1,10) = 7.74$, $P = 0.019$; $t = 60$, $P = 0.024$]. We also observed a significant effect of 20-day SERT treatment (vs. VEH) to enhance the glucagon response to SH [$F(1,11) = 8.5$, $P = 0.014$; $t = 60$, $P = 0.014$; $t = 120$, $P = 0.033$]. Finally, there was no significant effect of SERT treatment on ACTH [$F(1,11) = 3.88$, $P = 0745$] or corticosterone [$F(1,11) = 3.54$, $P = 0.0865$] responses to SH.

Likewise, in the RH treatment group, we also observed a main treatment effect of SERT to increase epinephrine secretion in 20-day RH SERT- vs. RH VEH-treated rats [$F(1,18) = 9.82$,

$P = 0.005$; Table 3]. Post hoc analysis revealed a significant increase in epinephrine in the RH SERT vs. RH VEH treatment group at $t = 60$ ($P = 0.001$), with a trend for epinephrine to be elevated at $t = 120$ ($P = 0.09$). The epinephrine response in RH SERT-treated rats was completely corrected, such that it was not statistically different from VEH-treated rats experiencing their first bout of hypoglycemia [SH: $F(1,16) = 3.00$, $P = 0.1024$; Table 3]. There was also a significant main effect of SERT treatment to increase norepinephrine levels in RH SERT- vs. RH VEH-treated rats [$F(1,18) = 8.09$, $P = 0.01$; $t = 60$, $P = 0.002$; $t = 120$, $P = 0.033$]. However, unlike SH SERT-treated rats, there was no significant difference in glucagon in RH SERT- vs. RH VEH-treated rats [$F(1,22) = 1.00$, $P = 0.322$]. Last, we did not observe any treatment effect of chronic SERT (vs. VEH) on ACTH [$F(1,23) = 2.85$, $P = 0.105$] or corticosterone [$F(1,25) = 0.008$, $P = 0.931$] levels in RH rats.

Feeding data for the chronic study are provided in Table 4. Two-hour food intake measured immediately following the SAL control infusion was similar between VEH- and SERT-treated rats [$F(1,9) = 0.036$, $P = 0.853$]. Hypoglycemia-stimulated food intake, measured at the termination of the 2-h insulin infusion, was similar in both VEH- and SERT-treated rats subjected to SH [$F(1,13) = 0.045$, $P = 0.835$] and RH [$F(1,23) = 0.011$, $P = 0.917$].

DISCUSSION

Our findings reveal that continuous treatment with the SSRI sertraline enhances key hormonal CRRs to a single bout of hypoglycemia in normal nondiabetic rats. The degree of enhancement of CRRs was dependent upon the duration of sertraline treatment. Six-day sertraline treatment potentiated adrenomedullary responses specifically, as there was no effect of the SSRI on hypoglycemia-induced norepinephrine, glucagon, ACTH, corticosterone, or feeding responses. In response to a longer duration of sertraline (20-day) treatment, however, multiple CRRs were affected. Hypoglycemia-induced epinephrine, norepinephrine, and glucagon responses were all significantly elevated above vehicle-treated controls. In this study, we also implemented our rodent model of acute recurrent hypoglycemia that, similarly to the human syndrome of HAAF (9), results in blunted epinephrine, glucagon, and norepinephrine CRRs (14). Here, we report that rats subjected to recurrent hypoglycemia and either duration of sertraline treatment (6 or 20 days) had a robust adrenomedullary response, unlike their vehicle-treated counterparts. Importantly, the epinephrine response in sertraline-treated recurrent hypoglycemic rats was similar to vehicle-treated rats experiencing their first bout of hypoglycemia; thus sertraline treatment completely prevented the impaired epinephrine response. That sertraline treatment rescued the impaired epinephrine response in recurrent hypoglycemic rats suggests that serotonergic mechanisms may contribute to the development of defective hypoglycemia-induced adrenomedullary secretion, a component of HAAF.

Collectively, our findings from both 6- and 20-day treatment studies clearly demonstrate an enhancement of hormonal CRRs under conditions of single or recurrent hypoglycemia. Similarly, in preliminary studies, Briscoe et al. (7) reported a significant elevation in hormonal CRRs under hyperinsulinemic hypoglycemic conditions in response to 6 wk of SSRI treatment in nondiabetic human subjects. Although some clinical literature suggests an association between SSRI therapy and inadvertent hypoglycemia, our data in rats and preliminary data from others in humans (7) do not support a direct action of sertraline in causing hypoglycemia or impairing hypoglycemia hormonal counterregulation. It is important to acknowledge that our studies were carried out in nondiabetic rats. Thus, it is possible that complex interactions exist among diabetic and depressive states, exogenous insulin delivery, and SSRI pharmacology that may exacerbate the risk of hypoglycemia but were not addressed in the present study.

Treatment with the SSRI sertraline may have affected insulin sensitivity, thereby impacting our findings in the present study. Moore et al. (34) reported that intraportal delivery of the SSRI fluvoxamine caused a rapid and significant elevation in net hepatic glucose uptake and increased hepatic glycogen levels in the presence of hyperinsulinemia but not euinsulinemia. Similarly, intraportal infusion of serotonin (35), or the serotonin precursor 5-hydroxytryptophan (36), has also been shown to enhance net hepatic glucose uptake in conscious dogs. Multiple serotonin receptor subtypes are expressed in the liver (19); thus, it is possible that there is a direct effect of serotonin to enhance hepatic insulin sensitivity. Furthermore, sertraline or fluvoxamine treatment in mice was shown to reduce plasma glucose without changing insulin levels (12), and in overweight patients with type 2 diabetes, 4-wk fluoxetine treatment improved insulin-mediated glucose disposal independently of weight loss (31). An SSRI-induced enhancement of insulin sensitivity, in conjunction with the frequent injections of exogenous insulin required for tight glycemic control in type 1 diabetes, may induce transient yet significant reductions in glucose that would otherwise not occur under conditions of physiological insulin release. In the present study, indexes of insulin sensitivity were not measured. Thus, we cannot rule out the possibility that a sertraline-induced enhancement in insulin sensitivity contributed to our findings. Further studies aimed at directly evaluating the effects of SSRIs on insulin secretion and sensitivity are required to fully understand this issue, especially in light of recent in vitro findings demonstrating that SSRIs, including sertraline, inhibit insulin signaling and contribute to cellular insulin resistance (23).

A consistent finding in the present study was the sertraline-induced enhancement of the adrenomedullary response to hypoglycemia. This effect of sertraline was observed only during hypoglycemia stress; basal epinephrine levels were not affected. This specific hypoglycemia-induced enhancement of epinephrine secretion was evident under both 6- and 20-day sertraline treatment conditions. That epinephrine secretion in sertraline-treated rats was enhanced only under conditions of hypoglycemia stress may reflect the selective innervation and recruitment of specific subpopulations of adrenal chromaffin cells in response to various stressful stimuli (3). Numerous observations have been made regarding serotonergic effects on adrenomedullary activation. Systemic or central delivery of $5HT_{1A}$, $5HT_{1C}$, or $5HT_2$ receptor agonists increases epinephrine levels in a dose-dependent manner (4,22). Although the specific central site(s) of action cannot be determined from the present study, a select population of serotonin neurons in the hindbrain is a potential neuroanatomical target. Caudal hindbrain serotonin neurons project to the intermediolateral cell column of the spinal cord, where they synapse on sympathetic preganglionic neurons innervating the adrenal medulla (47). This population of serotonin neurons expresses key glucose-sensing proteins (30), and direct application of the glucoprivic agent 5-thioglucose into this hindbrain site stimulates adrenomedullary secretion, glucagon, and corticosterone secretion and elicits a robust feeding response (2,40). Thus, it is possible that SSRI treatment modulated the responsiveness and/or sensitivity of these putative serotonin glucose-sensing neurons that control hormonal CRRs to hypoglycemia.

Although the mechanism of action of SSRIs is usually thought to involve an increase of the synaptic concentration of serotonin secondary to blockade of its reuptake by nerve terminals, it is also possible that nonneuronal mechanisms contribute. Sertraline treatment may have potentiated hypoglycemia-induced epinephrine secretion by a direct action in the adrenal medulla. Serotonin is present in ~75% of rat adrenal chromaffin cells, where it is localized with both epinephrine and the enzyme phenylethanolamine *N*-methyltransferase (18). Both serotonin reuptake transporter mRNA (6) and protein (44) are also localized in adrenal medullary chromaffin cells. Since tryptophan hydroxylase, the rate-limiting enzyme in serotonin synthesis, is not present in the adrenal medulla, it is thought that serotonin is captured from the blood and accumulates in chromaffin cells via the serotonin reuptake transporter (44). In line with this idea, genetic knockout mice lacking the transporter exhibit a significant

reduction in adrenal medullary serotonin content. Interestingly, mice lacking the serotonin reuptake transporter also exhibit an exaggerated epinephrine response to stress (48). Similar to genetic transporter knockout, we found that pharmacological blockade of the transporter with the SSRI sertraline enhanced the epinephrine response to hypoglycemia stress. Since we also found that sertraline treatment corrected the defective epinephrine response observed with recurrent hypoglycemia, further study will be necessary to determine whether repeated hypoglycemia stress induces changes in the adrenal medullary serotonergic system that contributes to the development of sympathoadrenal impairment.

In this study, we identified distinct effects of sertraline treatment that were expressed only in response to a longer duration (20 days) of drug exposure. These differential findings may be related to the time course of SSRI-induced changes in serotonergic neurotransmission or secondary changes downstream from serotonergic signaling. Acutely, SSRIs block the reuptake activity of the serotonin transporter, leading to initial increases in synaptic levels of serotonin (32). However, with >15 days of SSRI treatment, long-term adaptive changes in serotonin neurotransmission become apparent (5,5a). These changes are brain site specific and include reduced transporter binding and function, decreased transporter mRNA, reduced serotonin clearance, and downregulation in postsynaptic receptors (5,37). Our findings suggest that the sertraline-induced enhancement of the adrenomedullary response is not mediated by the long-term adaptive changes in serotonergic neurotrans-mission, since an increased epinephrine response was evident after just 6 days of sertraline treatment. The enhancement of the glucagon response to single hypoglycemia observed with 20-day, but not 6-day, sertraline treatment may be secondary to the overall heightened sympathoadrenomedullary responses that were observed in the 20-day sertraline-treated rats.

Activation of specific serotonergic receptor subtypes stimulate both the hypothalamic-pituitary-adrenal (HPA) axis and epinephrine secretion (22). In the present study, however, only hypoglycemia-induced epinephrine secretion was enhanced in response to 6- or 20-day sertraline treatment. It is well established that serotonin regulates HPA axis responsiveness. Serotonin precursors and receptor agonists, such as those targeting $5HT_{1A}$, $5HT_{1C}$, or $5HT_{2A}$ receptors, stimulate ACTH and corticosterone (4,33). A single injection of the SSRI citalopram also produces dose-dependent increases in ACTH, corticosterone, and c-*fos* protein in the paraventricular nucleus (20). However, SSRI-induced HPA axis activation and c-*fos* expression become blunted with daily SSRI treatment (20). Thus, it is not surprising that we did not observe any effect of continuous 6- or 20-day sertraline treatment on basal or hypoglycemia-induced ACTH or corticosterone responses. The selective effect of sertraline on hypoglycemia-induced adrenomedullary activation, without any alterations in ACTH or corticosterone, may suggest that perhaps sertraline-induced changes in the adrenal medulla contributed significantly to our findings.

In the present study, there was no effect of 6- or 20-day sertraline treatment on body weight. In contrast, previous studies have demonstrated that SSRIs administered for extended periods of time significantly reduce both body weight and caloric intake (17,21) This discrepancy may be due to the specific SSRIs used and their varying potencies, dose, route, and duration of administration. Studies reporting significant reductions in body weight and food intake also used higher doses and/or intraperitoneal route of chronic, continuous SSRI administration (17,21). Importantly, higher SSRI doses and/or intraperitoneal administration has been shown to induce gastroinstestinal pathologies, liver dysfunction, and kidney damage that may have induced nonspecific malaise, thereby contributing to the anorectic effect of SSRIs (21).

Although we observed selective and significant effects of sertraline on hypoglycemia hormonal CRRs, the feeding response to hypoglycemia, an important behavioral CRR, was similar between sertraline and vehicle-treated rats in all of the experimental groups. It is well

documented that administration of SSRIs results in reduced food intake (17,21,36). Additionally, a single injection of sertraline has been shown, in one study, to reduce food intake in rats deprived of food and water for 24 h (24). In the present study, it is evident that the well-characterized anorectic action of SSRIs had no effect on the powerful feeding response elicited by glucose deficit. Thus, chronic blockade of the serotonin reuptake transporter does not appear to modulate the neural circuitry required for the feeding response elicited by hypoglycemia. This may, in part, be due to the fact that a critical component of the neural circuitry mediating this response includes a select population of rostrally projecting hindbrain catecholamine neurons (39), whose activity and/or function may not be affected by SSRIs.

In conclusion, SSRI treatment, lasting for 6 or 20 days, results in a selective enhancement of key hormonal CRRs to a single bout of hypoglycemia and restores the defective adrenomedullary response observed in our acute rodent model of HAAF. Thus, these findings rule out a potential mechanism, impaired hormonal CRRs, for explaining the association between SSRI therapy and hypoglycemia in clinical case studies. Although these studies were carried out in normal nondiabetic rats, they contribute importantly to our understanding of how SSRI treatment impacts normal physiological responses to hypoglycemia and abnormal responses under conditions of recurrent hypoglycemia.

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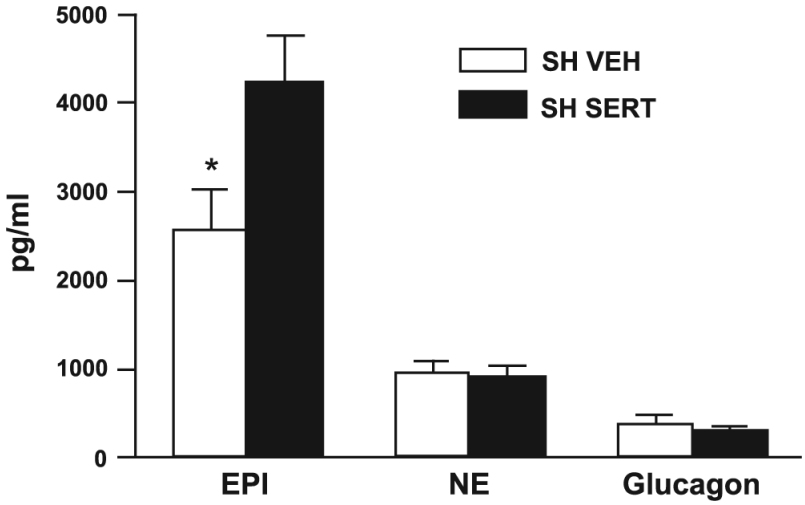

Fig. 1.

Six-day sertraline (SERT) treatment specifically enhances the epinephrine (EPI) response to single hypoglycemia (SH) at $t = 120$. *$P < 0.05$ vs. 6-day SH vehicle (VEH)-treated rats. See Table 2 for group sizes. Values are shown as means ± SE. NE, norepinephrine.

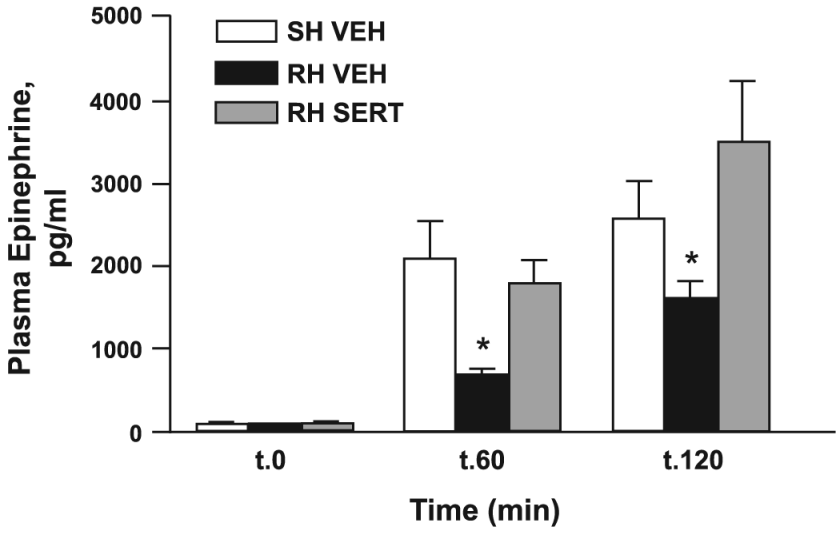

Fig. 2.

Six-day SERT treatment prevents the impaired EPI response observed in VEH-treated rats exposed to recurrent hypoglycemia (RH). *$P < 0.05$ vs. RH SERT and SH VEH. See Table 2 for group sizes. Values are shown as means ± SE.

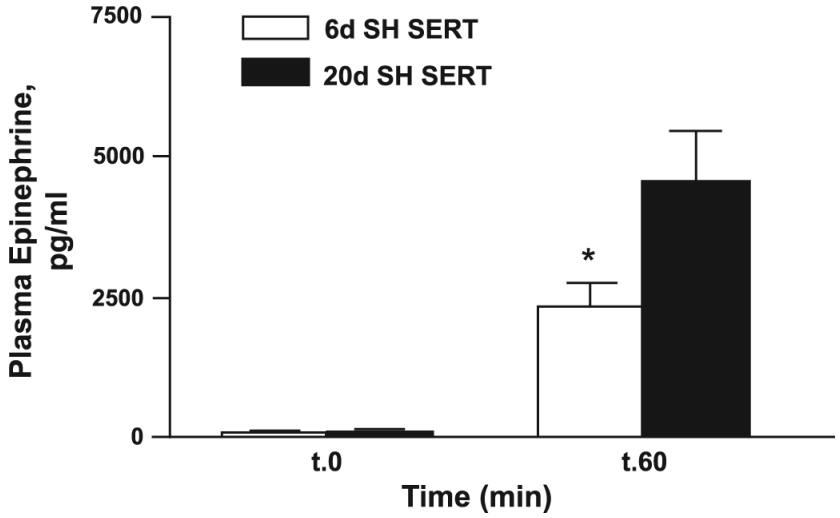

Fig. 3.

The EPI response to SH is significantly enhanced at $t = 60$ in 20-day vs. 6-day SERT-treated rats. $*P < 0.05$ vs. 20-day SH SERT treatment. See Tables 2 and 3 for group sizes. Values are shown as means ± SE.

Table 1

Body weight measurements on day 1: 6- and 20-day study

Experimental Group	VEH	SERT	*P*
	g		
6-Day study			
SAL	376±5	369±6	0.351
SH	391±14	370±5	0.130
RH	381±10	370±8	0.233
20-Day study			
SAL	446±7	460±8	0.217
SH	440±9	444±11	0.579
RH	445±6	448±7	0.709

Values are means ± SE. VEH, vehicle; SERT, sertraline; SAL, saline; SH, single hypoglycemia; RH, recurrent hypoglycemia.

Table 2

Plasma glucose and hormone responses: 6-day study

Time, min	Glucose, mg/dl	Glucagon	EPI	NE	ACTH	Corticosterone, ng/ml
			pg/ml			
			SAL control, VEH (n = 10)			
0	117±4	73±12	75±11	318±32	18±3	31±10
60	116±4	65±13	74±13	258±25	31±10	57±18
120	108±5	70±9	80±20	359±70	20±2	56±16
			SAL control, SERT (n = 8)			
0	112±3	72±15	70±9	203±24	18±3	13±3
60	101±3	54±5	68±12	319±35	30±5	52±15
120	104±4	56±10	109±26	362±80	20±5	35±22
			SH, VEH (n = 10)			
0	117±4	50±9	107±19	311±50	14±2	21±5
60	42±3	245±30	2,081±436	766±61	151±35	193±25
120	34±3	290±53	2,573±448	972±118	166±17	292±14
			SH, SERT (n = 11)			
0	116±6	63±8	100±16	325±32	20±3	27±9
60	42±3	245±30	2,329±410	675±111	180±38	168±13
120	34±3	290±53	4,202±545*	918±113	211±26	250±16
			RH, VEH (n = 10)			
0	122±4	64±12	107±17	313±40	12±2	23±5
60	43±3	152±22*	678±73*	663±61	120±16	195±18
120	32±3	167±32*	1,611±211*	894±80	131±18	247±14
			RH, SERT (n = 13)			
0	116±4	53±8	112±17	298±37	16±1	28±4
60	44±3	185±36	1,794±276†	662±56	161±34	200±25
120	37±4	170±24	3,490±735†	799±100	132±23	247±17

Values are means ± SE. EPI, epinephrine; NE, norepinephrine.

*$P < 0.05$ vs. SH vehicle control

†$P < 0.05$ vs. RH vehicle control.

Table 3

Plasma glucose and hormone responses: 20-day study

Time, min	Glucose, mg/dl	Glucagon	EPI	NE	ACTH	Corticosterone, ng/ml
			pg/ml			
			SAL control, VEH (n = 8)			
0	114±5	101±10	98±19	325±17	21±2	40±9
60	106±6	109±11	116±45	368±34	20±3	37±11
120	105±4	113±7	105±27	485±47	22±2	49±13
			SAL control, SERT (n = 7)			
0	104±2	97±11	82±5	279±29	27±1	42±5
60	100±3	101±14	134±37	395±72	25±2	40±9
120	98±2	102±12	106±22	430±77	26±2	60±16
			SH, VEH (n = 7)			
0	117±5	95±12	134±19	334±41	19±2	37±11
60	34±2	233±26	1,506±151	547±77	113±38	182±27
120	38±3	265±41	3,054±644	757±130	174±43	275 + 20
			SH, SERT (n = 7)			
0	114±6	100±8	144±13	353±49	23±3	46±11
60	38±3	429±7*	4,561±89*	942±126*	180±37	247±16
120	30±3	492±8*	5,641±1,02*	1,094 + 111	160±19	293±12
			RH, VEH (n = 8)			
0	118±4	92±12	129±30	382±58	16±3	38±7
60	41±3	171±17	645±135	514±68	106±11	200±22
120	34±2	234±45	2,287±639	746±132	114±14	298±16
			RH, SERT (n = 10)			
0	114±3	103±6	159±15	354±29	21±2	43±5
60	38±1	318±76	3,187±488†	1,030±107†	166±38	222±15
120	31±1	294±29	3,771±533	1,200±133†	161±19	285±17

Values are means ± SE.

*$P < 0.05$ vs. SH vehicle control

†$P < 0.05$ vs. RH vehicle control.

Table 4

Two-hour food intake in response to day 2 hypoglycemia or saline

Treatment	6-Day VEH	6-Day SERT	20-Day VEH	20-Day SERT
SAL	2.4±0.5	1.7±0.3	3.0±0.5	2.6±0.8
SH	4.0±0.4	4.1±0.5	6.0±0.5	5.9±0.4
RH	4.6±0.5	4.9±0.4	5.4±0.6	5.8±0.4

Values are means ± SE and in g.