OBJECTIVE—Previous work has demonstrated that chronic administration of the serotonin reuptake inhibitor (SSRI) fluoxetine augments counterregulatory responses to hypoglycemia in healthy humans. However, virtually no information exists regarding the effects of fluoxetine on integrated physiological counterregulatory responses during hypoglycemia in type 1 diabetes. Therefore, the specific aim of this study was to test the hypothesis that chronic use of the SSRI fluoxetine would amplify autonomic nervous system (ANS) counterregulatory responses to hypoglycemia in individuals with type 1 diabetes.

RESEARCH DESIGN AND METHODS—Eighteen type 1 diabetic patients (14 men/4 women aged 19–48 years with BMI 25 ± 3 kg/m² and A1C 7.0 ± 0.4%) participated in randomized, double-blind 2-h hyperinsulinemic (9 pmol·kg⁻¹·min⁻¹)-hypoglycemic clamp studies before and after 6 weeks of fluoxetine administration (n = 8) or identical placebo (n = 10). Glucose kinetics was determined by 3-tritiated glucose. Muscle sympathetic nerve activity (MSNA) was determined by microneurography.

RESULTS—Hypoglycemia (2.8 ± 0.1 mmol/l) and insulinemia (646 ± 52 pmol/l) were similar during all clamp studies. ANS, neuroendocrine, and metabolic counterregulatory responses remained unchanged in the placebo group. However, fluoxetine administration significantly (P < 0.05) increased key ANS (epinephrine, norepinephrine, and MSNA), metabolic (endogenous glucose production and lipolysis), and cardiovascular (systolic blood pressure) counterregulatory responses during hypoglycemia.

CONCLUSIONS—This study has demonstrated that 6-week administration of the SSRI fluoxetine can amplify ANS and metabolic counterregulatory mechanisms during moderate hypoglycemia in patients with type 1 diabetes. These data also suggest that the use of fluoxetine may be useful in increasing epinephrine responses during hypoglycemia in clinical practice. Diabetes 57:3315–3322, 2008

Selective serotonin reuptake inhibitors (SSRIs) are effective drugs for the treatment of depressive disorders associated with reduced serotonin function. Serotonergic neurons play an important role in the regulation of neuroendocrine function carried out via both sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) pathways.
consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board.

All study patients were asked to avoid any exercise and consume their usual weight-maintaining diet for 3 days before each experiment. All patients performed intensive home blood glucose monitoring (i.e., at least four glucose tests per day) and were asked to avoid hypoglycemia for at least 5 days before a study. On the day before a study, intermediate or long-acting insulin was discontinued and replaced by injections of regular insulin before breakfast and lunch. Each subject was admitted to the Vanderbilt General Clinical Research Center (GCRC) at 5:00 p.m. on the evening before an experiment. At this time, two intravenous canulae were inserted under 1% lidocaine local anesthesia. One canula was placed in a retrograde fashion into a vein on the back of the hand. This hand would be placed in a heated box (55–60°C) during the study. After the other canula was placed in the contralateral arm for infusions. Patients then received a standardized evening meal, and a continuous low-dose infusion of insulin was started to normalize plasma glucose. The insulin infusion was adjusted overnight to maintain blood glucose between 4.4 and 7.2 mmol/l.

Hypoglycemia experiments. After an overnight 10-h fast at 0 min, a primed (18-pCi) continuous infusion (0.18 μCi/min) of high-performance liquid chromatography (HPLC)-purified [3-3H]glucose (11.5 mCi.1 mmol−1; Perkin Elmer Life Sciences, Boston, MA) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA). A period of 90 min was allowed to elapse followed by a 30-min basal control period and a 120-min hyperinsulinemic-hypoglycemic experimental period. An insulin infusion solution was prepared with normal saline containing 3% (vol/vol) of the subject’s own plasma. At time 120 min, a primed constant (0.00 pmol·kg−1·min−1) infusion of insulin (Human Regular Insulin; Eli Lilly, Indianapolis, IN) was started at a rate of 240 nmol/min. The rate of glucose infusion was 0.06 mmol/min and the glucose nadir (2.8 mmol/l) was achieved using a modification of the glucose clamp technique (14). During the clamp period, plasma glucose was measured every 5 min, and a 20% dextrose infusion was used to adjust for a subject’s 1.3-s vascular response (15). The contralateral canula was placed in the contralateral arm for infusions. Patients then received a standardized evening meal, and a continuous low-dose infusion of insulin was started to normalize plasma glucose. The insulin infusion was adjusted overnight to maintain blood glucose between 4.4 and 7.2 mmol/l.

Study medication. After the initial 1-day clamp study, volunteers were given a 1-week medication supply of either fluoxetine or placebo for 6 weeks. The fluoxetine dose was as follows: 20 mg/day during week 1, 40 mg/day during week 2, 60 mg/day during week 3, and 80 mg/day during weeks 4–6. The study was carried out in a double-blind fashion with volunteers and investigators blinded to the treatment group assigned. Stratified blocked randomization was performed by the Vanderbilt Investigational Drug Pharmacy. The subjects were stratified according to sex because it is known to affect the counterregulatory response (16). Randomization was performed within each sex, and blocks of two were used to ensure an equal number of men and women in the placebo and fluoxetine treatment groups.

During the 6-week treatment period, volunteers came to the GCRC once a week for compliance and adverse events. Compliance was determined via a pill count and a blood draw to measure serum fluoxetine levels. Two subjects (1 man/1 woman) in the fluoxetine group withdrew from the study because of side effects. The withdrawals occurred early at the 20-mg dose and were described as feelings of tiredness and nonspecific malaise. In the remaining subjects, fluoxetine was very well tolerated, with no reports of side effects. After taking either placebo or fluoxetine for 6 weeks, subjects underwent another single-day hyperglycemic clamp study as previously described. Thus, 18 subjects (8 fluoxetine/10 placebo) completed both hyperglycemic clamp studies. On completion of this second 1-day study, subjects were tapered off the study medication (placebo or fluoxetine). Those receiving fluoxetine were given it for 1 week at 40 mg/day and 1 week at 20 mg/day. Once subjects finished the medication, they were unblinded as to the medication they had taken.

Direct measurement of MSNA. MSNA was recorded from the peroneal nerve at the level of the fibular head and popliteal fossa (17,18). Nerve activity was recorded on a PC-based Windaq data acquisition system at 1,000 Hz through a 1-mm channel-1 (DATAQ Instruments, Akron, OH). Five-minute Windaq files were analyzed with a MatLab GUIDE interface (to adjust for an individual’s I.3-s nerve burst delay from a once-removed-R-R interval, automatically detected by pulse synchronicity, a 2:1 signal/noise ratio, and wave-form shape). Further criteria for the MSNA recordings were that 1) the waveforms produced muscle twitches but not paresthesia, 2) nerve activity increased during phase II of the Valsalva maneuver (hypotensive phase) and was suppressed during phase IV (blood pressure overshoot), and 3) nerve activity increased in response to hand held expiration.

Tracer calculations. Rates of glucose appearance (Ra), endogenous glucose production (EGP), and glucose utilization were calculated according to the methods of Wall et al. (19). EGP was calculated by determining the total Ra (this comprises EGP and any exogenous glucose infused to maintain the steady-state glucose levels) and subtracting it from the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative because underestimates of total Ra and rate of glucose disposal (Rd) can be obtained. The use of a highly purified tracer and taking measurements under steady-state conditions (i.e., constant specific activity) in the presence of low glucose flux eliminates most, if not all, of the problems. In addition, to maintain a constant specific activity, isotope delivery was increased commensurate with increases in exogenous glucose infusion. During these studies, only glucose flux results from the steady-state basal and the final 30-min periods of the hyperglycemic clamps are reported.

Analytical methods. Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Glucagon was measured by radioimmunoassay (RIA) with an interassay and coefficient of variation (CV) of 12% (20). Insulin was measured as previously described (21) with an interassay CV of 9%. Catecholamines were determined by HPLC (22) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. Cortisol was assayed using the Clinical Assays Gamma Coat RIA kit with an interassay CV of 6%. Growth hormone was determined by RIA (23) with a CV of 8.6%. Pancreric polypeptide was measured by RIA using the method of Hagopian et al. (24) with an interassay CV of 8%. Lactate, pyruvate, alanine, and glycerol were measured in deproteinized whole blood using the method of Lloyd et al. (25). Nonesterified fatty acids (NEFAs) were measured using the WAKO kit adopted for use on a centrifugal analyzer (26). Fluoxetine and norfluoxetine were determined by gas chromatography with electron capture detection based on a modification described by Torok-Both et al. (27).

Blood for hormones and intermediary metabolites was drawn twice during the control period and every 15 min during the experimental period. Cardiovascular parameters (pulse, systolic, diastolic, and mean arterial pressure) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min throughout each study starting at 80 min.

Hypoglycemic symptoms were quantified using a previously validated semiquantitative questionnaire (28). Each individual was asked to rate his/her hypoglycemic symptoms (including hypoglycemia, malingering, and the like) at the beginning and end of each 30-min period of the hypoglycemic clamp studies before the clamp (fluoxetine, 74.2 ± 42.1 rating units). The ratings were 0 to 100, where 0 was no symptoms and 100 was the most symptoms. Hypoglycemic symptoms were summed over the 2-h period and were described as feelings of tiredness and nonspecific malaise. In the remaining subjects, fluoxetine was very well tolerated, with no reports of side effects. After taking either placebo or fluoxetine for 6 weeks, subjects underwent another single-day hyperglycemic clamp study as previously described. Thus, 18 subjects (8 fluoxetine/10 placebo) completed both hyperglycemic clamp studies. On completion of this second 1-day study, subjects were tapered off the study medication (placebo or fluoxetine). Those receiving fluoxetine were given it for 1 week at 40 mg/day and 1 week at 20 mg/day. Once subjects finished the medication, they were unblinded as to the medication they had taken.

Results. Basal plasma glucose levels were 6.3 ± 0.3 and 5.6 ± 0.2 mmol/l in the clamps before fluoxetine and before placebo. Basal glucose levels were similar (6.1 ± 0.3 and 5.5 ± 0.3 mmol/l) after 6 weeks of fluoxetine or placebo. Plasma glucose levels reached steady state by 30 min, and equivalent hypoglycemia (2.8 ± 0.1 mmol/l) was maintained during all clamp procedures (Fig. 1). Basal and steady-state insulin levels for both fluoxetine and placebo groups were similar during studies before the clamp (fluoxetine, 120 ± 18 and 660 ± 24 pmol/l, and placebo, 126 ± 30 and 648 ± 54 pmol/l) and after clamp (fluoxetine 120 ± 18 and 630 ± 42 pmol/l, and placebo, 144 ± 30 and 667 ± 30 pmol/l) (Fig. 2). AIC levels were unchanged during fluoxetine (7.0 ± 0.3 to 6.8 ± 0.3%) and placebo (6.9 ± 0.3 to 6.9 ± 0.3%) administration. Similarly, weight was constant during both fluoxetine (74.2 ± 13 to 73 ± 7 ± 14.3 kg) and placebo (77.5 ± 9 to 77.4 ± 9 kg) administration. Mean fluoxetine
and norfluoxetine levels at the end of the study were 274.13 ± 45 and 188.38 ± 34 ng/ml, respectively, in the SSRI group and were undetectable in the placebo group.

**Neuroendocrine counterregulatory hormones.** Epinephrine responses were significantly higher \( (P < 0.05) \) during the final 30 min of hypoglycemia after fluoxetine \( (5,436 ± 808 \text{ pmol/l}) \) compared with before treatment \( (3,815 ± 841 \text{ pmol/l}) \) and after placebo \( (3,198 ± 791 \text{ pmol/l}) \) hypoglycemic clamp. Epinephrine responses were similar during the final 30 min of hypoglycemia before and after placebo (Fig. 2).

Norepinephrine responses were significantly higher \( (P < 0.05) \) during the final 30 min of after fluoxetine \( (2.3 ± 0.3 \text{ nmol/l}) \) compared with before treatment \( (1.7 ± 0.2 \text{ nmol/l}) \) and after placebo \( (1.6 ± 0.2 \text{ nmol/l}) \). Norepinephrine responses were similar during the final 30 min of hypoglycemia during the studies before and after placebo (Fig. 2).

Cortisol levels were increased \( (P < 0.05) \) in the after-fluoxetine versus the after-placebo groups \( (800 ± 55 \text{ vs. } 635 ± 83 \text{ nmol/l}) \). Cortisol levels remained unchanged during placebo administration.

Peak pancreatic polypeptide levels during hypoglycemia were practically unchanged \( (135 ± 37 \text{ vs. } 138 ± 27 \text{ nmol/l}) \) after fluoxetine administration compared with values before treatment, respectively. Pancreatic

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**FIG. 1.** Plasma glucose \( (A) \) and insulin \( (B) \) concentrations (means ± SE) during hypoglycemic clamp studies in 18 individuals (14 men/4 women) with type 1 diabetes before and after 6 weeks of fluoxetine or placebo administration. pre-SSRI, ○; post-SSRI, ■; pre-placebo, △; post-placebo, ▼.

**FIG. 2.** Mean plasma epinephrine \( (A) \), cortisol \( (B) \), and norepinephrine \( (C) \) levels (means ± SE) during the basal period and the final 30 min of hypoglycemic clamp studies in 18 individuals (14 men/four women) with type 1 diabetes before and after 6 weeks of fluoxetine or placebo administration. *Plasma epinephrine, norepinephrine, and cortisol levels are significantly increased \( (P < 0.05) \) following fluoxetine. pre-SSRI, ○; post-SSRI, ■; pre-placebo, △; post-placebo, ▼.
polypeptide levels were also similar for the control group after placebo (115 ± 35 nmol/l) versus before placebo (121 ± 26 nmol/l) during the final 30 min of hypoglycemia (Fig. 3).

Glucagon responses were similar during hypoglycemia in all groups (before/after fluoxetine and before/after placebo) (Fig. 3). Fluoxetine and placebo had similar effects on growth hormone responses during hypoglycemia. Growth hormone levels during the studies before and after fluoxetine were 41 ± 7 vs. 36 ± 6 ng/l, respectively, and were 41 ± 10 vs. 34 ± 7 ng/l for the before- and after-placebo groups, respectively (Fig. 3).

MSNA. MSNA increased by a significantly greater amount ($P < 0.05$) during hypoglycemia after fluoxetine (16 ± 3 bursts/min) versus both after placebo (10 ± 2 bursts/min) and before fluoxetine (5 ± 2 bursts/min). There were no differences in the after-placebo versus before-placebo responses (Fig. 4).

FIG. 3. Mean plasma pancreatic polypeptide (A), glucagon (B), and growth hormone (C) levels (means ± SE) during the basal period and the final 30 min of hypoglycemic clamp studies in 18 individuals (14 men/four women) with type 1 diabetes before and after 6 weeks of fluoxetine or placebo administration.

FIG. 4. $\Delta$MSNA (A) and total symptom (B) responses during the final 30 min of hypoglycemic clamp studies in 18 individuals (14 men/4 women) with type 1 diabetes before and after 6 weeks of fluoxetine or placebo administration. Data are means ± SE. *MSNA responses are significantly increased ($P < 0.05$) following fluoxetine.
Glucose kinetics. Glucose specific activity (disintegrations per minute per milligram) was in a steady state during the basal period and the final 30 min of all hyperinsulinemic-hypoglycemic clamps (Table 1). EGP after fluoxetine (11 ± 1 µmol·kg⁻¹·min⁻¹) was significantly increased (P < 0.05) during the final 30 min of hypoglycemia compared with before fluoxetine (5.1 ± 1 µmol·kg⁻¹·min⁻¹), before placebo (2.2 ± 1.6 µmol·kg⁻¹·min⁻¹), and after placebo (5.0 ± 2.2 µmol·kg⁻¹·min⁻¹). Glucose infusion rates were 14.3 ± 6.1 and 8.9 ± 3.9 µmol·kg⁻¹·min⁻¹ after placebo and after fluoxetine, respectively. Glucose Rd during the last 30 min of hypoglycemia were 19 ± 2.8 and 19.8 ± 3.3 after placebo and after fluoxetine, respectively (Fig. 5).

Intermediary metabolism. Baseline glycerol, lactate, β-hydroxybutyrate, NEFA, and alanine levels were similar among groups (Table 2). Glycerol levels were significantly greater (P < 0.05) after fluoxetine (115 ± 18 µmol/l) compared with after placebo (83 ± 16 µmol/l) or before fluoxetine (73 ± 5 µmol/l). There was no difference in the increase of glycerol in the studies before and after placebo (Table 2).

There was an increase in NEFA levels during hypoglycemia after fluoxetine (182 ± 64 µmol/l) compared with after placebo (91 ± 25 µmol/l) and before fluoxetine (93 ± 24 µmol/l) (P < 0.05) studies. There was no difference in the increase of NEFA levels during hypoglycemia in the studies before and after placebo. Blood lactate, alanine, and β-hydroxybutyrate levels were similar during the four series of hypoglycemic clamps and were unaffected by fluoxetine or placebo administration (Table 2).

Cardiovascular parameters. Basal heart rate and blood pressure were not different after a 6-week administration of fluoxetine. Heart rate was significantly higher (P < 0.05) during the final 30 min of groups after fluoxetine compared with before fluoxetine and after placebo (80 ± 4 vs. 74 ± 5 and 69 ± 5 bpm, respectively). Heart rate was similar in the control group (after placebo 69 ± 5 bpm vs. before placebo, 67 ± 8 bpm). Systolic blood pressure was significantly increased during hypoglycemia in the group after fluoxetine versus the before-fluoxetine and after-placebo groups (127 ± 4 vs. 113 ± 3 and 115 ± 6 mmHg, respectively; P < 0.05). There were no differences in systolic blood pressure in the placebo control group (after placebo 115 ± 6 mmHg vs. before placebo 119 ± 5 mmHg). Diastolic blood pressure was also increased after fluoxetine (P < 0.05) compared with after placebo and before fluoxetine (67 ± 2 vs. 61 ± 3 and 61 ± 1 mmHg, respectively; Table 3).

Symptom response. There were no differences in total symptom scores in the experimental group when compared with groups before fluoxetine or after placebo. Similarly, no significant differences occurred in the control group after placebo compared with the clamp study before placebo (Fig. 4). Both autonomic and neuroglycopenic symptoms scores were similar during hypoglycemia be-

**TABLE 1**

Glucose specific activity during the basal period and the final 30 min of hyperinsulinemic-hypoglycemic clamps in subjects with type 1 diabetes before and after 6 weeks of fluoxetine or placebo administration

|                      | Glucose specific activity (dpm/µmol) |
|----------------------|-------------------------------------|
|                      | −20 min | −10 min | 0 min | 90 min | 105 min | 120 min |
| Before fluoxetine    | 364 ± 22| 341 ± 25| 367 ± 18| 273 ± 22| 289 ± 13| 276 ± 12|
| After fluoxetine     | 334 ± 42| 353 ± 33| 359 ± 33| 262 ± 22| 260 ± 20| 247 ± 20|
| Before placebo       | 326 ± 18| 334 ± 24| 338 ± 19| 271 ± 22| 263 ± 17| 259 ± 19|
| After placebo        | 365 ± 24| 369 ± 22| 376 ± 24| 300 ± 20| 289 ± 18| 272 ± 18|

Data are means ± SE.
**TABLE 2**  
Plasma glycerol, lactate, β-hydroxybutyrate, NEFA, and alanine levels during the basal period and the final 30 min of hyperinsulinemic-hypoglycemic clamp studies in type 1 diabetes before and after 6 weeks of fluoxetine administration  

|                      | Basal period | Final 30 min |
|----------------------|--------------|--------------|
| Glycerol (μmol/l)    |              |              |
| Before treatment     | 58 ± 7       | 65 ± 8       |
| After fluoxetine     | 95 ± 25      | 110 ± 21*    |
| Before treatment     | 67 ± 15      | 74 ± 15      |
| After placebo        | 73 ± 11      | 80 ± 17      |
| Lactate (mmol/l)     |              |              |
| Before treatment     | 0.4 ± 0.04   | 0.75 ± 0.09  |
| After fluoxetine     | 0.3 ± 0.02   | 0.85 ± 0.15  |
| Before treatment     | 0.4 ± 0.04   | 0.75 ± 0.09  |
| After placebo        | 0.4 ± 0.05   | 0.84 ± 0.00  |
| β-Hydroxybutyrate (μmol/l) | 0.009 ± 0.089 | 0.05 ± 0.03 |
| NEFA (μmol/l)        |              |              |
| Before treatment     | 149 ± 38     | 93 ± 24      |
| After fluoxetine     | 165 ± 41     | 182 ± 64*    |
| Before treatment     | 131 ± 34     | 86 ± 22      |
| After placebo        | 212 ± 37     | 91 ± 24      |
| Alanine (μmol/l)     |              |              |
| Before treatment     | 0.23 ± 0.02  | 0.22 ± 0.02  |
| After fluoxetine     | 0.21 ± 0.02  | 0.21 ± 0.03  |
| Before treatment     | 0.21 ± 0.02  | 0.20 ± 0.02  |
| After placebo        | 0.24 ± 0.03  | 0.22 ± 0.02  |

Data are means ± SE. *Significantly increased responses during final 30 min of hypoglycemia after 6 weeks of fluoxetine administration (P < 0.05).

**DISCUSSION**  
This study has determined the effects of 6-week administration of the SSRI fluoxetine on counterregulatory responses to hypoglycemia in nondepressed patients with type 1 diabetes. Our results demonstrate that a period of 6 weeks of high-dose fluoxetine significantly increases sympathetic nervous system, HPA, and metabolic (EGP and lipolysis) counterregulatory responses in patients with long-duration type 1 diabetes.

Hypoglycemia remains the major barrier to even near normalization of glucose in patients with type 1 diabetes. We have recently reported the beneficial effects of 6 weeks' fluoxetine administration on amplifying counterregulatory mechanisms during hypoglycemia in nondiabetic individuals (9). In this present study, we have tested the hypothesis that 6 weeks' administration of fluoxetine will enhance counterregulatory responses during hypoglycemia in type 1 diabetes. Similar to studies in nondiabetic humans, this study has determined that fluoxetine can have marked effects in amplifying catecholamine responses and muscle sympathetic nervous activity during hypoglycemia. The increased sympathetic nervous system (SNS) activity also had significant effects on enhancing the key counterregulatory metabolic mechanisms of EGP and lipolysis.

In longer duration type 1 diabetes patients, epinephrine becomes the critical counterregulatory hormone in the defense against acute hypoglycemia. This is due to the fact that with increasing disease duration, the glucagon response to hypoglycemia is lost in type 1 diabetes (29). Unfortunately, in type 1 diabetic patients with repeated episodes of hypoglycemia and intensive glucose control, the epinephrine response to hypoglycemia is significantly reduced (30). The combination of absent glucagon and severely blunted epinephrine responses consequently results in a significant magnification of the risk for severe hypoglycemia (30). Perhaps the most notable finding from the present study was the striking increase in epinephrine responses during hypoglycemia following fluoxetine. Despite equivalent insulin and glucose levels during the hypoglycemic clamps, fluoxetine resulted in a 90% increase in epinephrine levels. In fact, fluoxetine increased the response of epinephrine to levels even higher than previously observed in nondiabetic individuals during similar conditions of hypoglycemia (31). The increased SNS response following fluoxetine also resulted in a marked increase in EGP and lipolysis. As can be observed from the placebo studies, EGP is typically suppressed during hypoglycemia in type 1 diabetes. Thus, the amplified (doubled) EGP response following fluoxetine represents a potentially important defense against hypoglycemia in type 1 diabetes. The increased lipolytic response following fluoxetine would also be expected to contribute to the elevated rates of EGP. Both glycerol, an important gluconeogenic precursor during hypoglycemia (32), and NEFA, a significant provider of energy for gluconeogenesis (32), were significantly increased. Rates of glucose uptake were unchanged during the series of hypoglycemic clamps. Previous studies have demonstrated that the dose-response effects of epinephrine to inhibit glucose uptake are relatively flat (33,34) in association with increasing the concentrations of the catecholamines from 600 to 1,000 pg/ml, producing limited actions. Similar to the findings in healthy humans, fluoxetine resulted in an increased response of cortisol during hypoglycemia. This also demon-
strates that the SSRI was having effects to activate multiple neural pathways within the central nervous system (7).

Accompanying the amplified SNS responses were significant increases in blood pressure and heart rate during hypoglycemia following fluoxetine. Somewhat surprisingly, there was no increase in symptom scores despite the significant increases in central ANS drive and the respective target organ responses (i.e., liver, adipose tissue, and heart) following fluoxetine. This apparent disassociation between neutral symptom responses and increases in other components of the sympathetic nervous system following fluoxetine was also clearly demonstrated in the previous study with nondiabetic individuals (9). These data further suggest that serotonergic pathways are involved in the generation of symptom responses during hypoglycemia. Recent work in humans has demonstrated that hippocampal and thalamic regions of the brain are activated during hypoglycemia (35). These areas are involved in mood and function known to be affected by SSRIs (36). We therefore hypothesize that fluoxetine exerted a relatively restraining effect on symptom generation while stimulating neurohumoral and cardiovascular sympathetic tone during hypoglycemia. Similar to our findings in nondiabetic individuals, despite the increased sympathetic nervous system drive and elevated epinephrine levels, fluoxetine had no effects on amplifying glucagon responses during hypoglycemia (9). This would add support to the hypothesis that there is an inherent B-cell defect restricting glucagon release during hypoglycemia in type 1 diabetes rather than simply just a loss of ANS input into the α-cells (37).

Another point that should be noted is that fluoxetine only amplified counterregulatory responses during hypoglycemia and did not increase basal homeostatic mechanisms. Thus, there were no differences in baseline cardiovascular, metabolic, and neuroendocrine parameters following fluoxetine or placebo. The lack of chronic effects of an SSRI to increase basal neuroendocrine activity before hypoglycemia was also reported by Sanders et al. (10) in their recent study using sertraline in nondiabetic conscious rats. We also found that chronic administration of the SSRI fluoxetine in nondiabetic individuals had no effects on increasing basal cardiovascular, ANS, and metabolic function. However, basal plasma cortisol (but not glucagon and growth hormone) was increased by fluoxetine in nondiabetic individuals.

Data are accumulating regarding possible mechanisms for SSRI enhancement of ANS and HPA axis responses during hypoglycemia. Activation of a number of serotonergic receptors (5HT1A, 5HT1C, 5HT2, and 5HT3) has been demonstrated to increase sympathetic nervous system outflow (5,6,38,39). Additionally, both systemic and central administration of SSRIs have specifically increased adrenal catecholamine and epinephrine release (7,8). Two recent studies have also demonstrated that chronic administration of different SSRIs can amplify counterregulatory responses during hypoglycemia in both healthy humans and conscious rats (9,10). The findings of increased cortisol and catecholamines cannot determine whether the SSRI was being sensed at central (i.e., brain), peripheral (i.e., adrenal gland), or even both sites to augment counterregulatory responses. However, the finding that MSNA was increased in the present study certainly indicates that central sensing and action of the SSRI were occurring. The effects of SSRIs on HPA responses during stress appear to be more complex. We have found that fluoxetine can increase plasma cortisol responses during hypoglycemia in both healthy and type 1 diabetes humans. Durand et al. (40) have also demonstrated that fluoxetine can amplify corticosteroid responses to stress in conscious rats. However, Sanders et al. (10), studying a different SSRI (sertraline) and species of rat (Sprague-Dawley), found no amplification of HPA axis responses during hypoglycemia. Thus, the physiological effects of SSRI may be different depending on the specific agent and experimental model under investigation.

The present study has provided an evaluation of the effects of fluoxetine on physiological responses during hypoglycemia in a group of metabolically well-controlled type 1 diabetic patients. These individuals typically have the highest prevalence of hypoglycemia and might be expected to benefit most from strategies aimed at improving ANS responses. Six-week administration of high-dose fluoxetine had marked effects in amplifying epinephrine and metabolic counterregulatory responses. However, the dose of fluoxetine used in this study is higher than the average dose of the drug used in clinical practice, ~33 mg/day (41). Fluoxetine was increased to the highest clinically approved dose of the drug because we wanted to ensure that the largest experimental signal was generated in these initial studies. Additionally, the subjects enrolled in our study were not depressed and did not have any major psychopathology. Thus, we cannot determine whether similar results would be obtained in depressed individuals. In addition, we did not formally assess whether fluoxetine would have any effects on cognitive function during hypoglycemia. This is an important clinical consideration and deserves further study. Overall, fluoxetine was well tolerated. Body weight and A1C were no different in the placebo or fluoxetine groups. Rates of hypoglycemia (albeit anecdotally reported by the patients) appeared to be unchanged (n = 3), improved (n = 3), or relatively improved (n = 2) (i.e., unchanged frequency of hypoglycemia with improvement in A1C of ≥0.8%). Therefore, we cannot determine whether fluoxetine increased sympathoadrenal responses by reducing episodes of antecedent hypoglycemia and/or by directly stimulating ANS responses during any given episode of hypoglycemia. Fluoxetine administration was started at 20 mg and increased in a stepwise fashion to 80 mg in an attempt to limit side effects of the drug. However, two subjects receiving fluoxetine withdrew early from the study. Both of these subjects were only receiving 20 mg of the drug and reported nonspecific side effects. Interestingly, the higher doses of the drug (60–80 mg/day) were very well tolerated, with no reports of unpleasant side effects. Thus, in the present study (and our previous study in nondiabetic individuals), the side effects with fluoxetine appeared early, dissipated after continued use, and were not dose related.

In summary, this study has demonstrated that 6 weeks’ administration of fluoxetine can markedly increase key sympathetic nervous system (epinephrine, norepinephrine, and muscle sympathetic nervous system), metabolic (EGP and lipolysis), and cardiovascular counterregulatory responses during clamped moderate (2.8 mmol/l) hypoglycemia in type 1 diabetes. The study also demonstrates that serotonergic mechanisms can play a significant role in regulating ANS and HPA physiological responses during hypoglycemia in type 1 diabetic individuals. In conclusion, the present results have provided novel findings demon-
stratizing that under the conditions of the present study, SSRIs (specifically fluoxetine) may provide beneficial ad- junct effects in amplifying epinephrine levels during hypoglycemia in type 1 diabetic individuals.

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