Effects of the Selective Serotonin Reuptake Inhibitor, Fluoxetine, on Counterregulatory Responses to Hypoglycemia in Individuals with T1DM

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Clinical trial reg. No. NCT00592670, clinicaltrials.gov.

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ABSTRACT

**Objective:** Previous work has demonstrated that chronic administration of the serotonin reuptake inhibitor (SSRI), fluoxetine, augments counterregulatory responses to hypoglycemia in healthy man. However, virtually no information exists regarding the effects of fluoxetine on integrated physiologic counterregulatory responses during hypoglycemia in type 1 diabetes mellitus (T1DM). Therefore, the specific aim of this study was to test the hypothesis that 6 weeks use of the SSRI, fluoxetine, would amplify autonomic nervous system (ANS) counterregulatory responses to hypoglycemia in individuals with T1DM.

**Research Design and Methods:** Eighteen T1DM (14M/4F) (age 19yrs - 48yrs, BMI 25±3 kg/m², and HbA1c 7.0±0.4%) participated in randomized, double-blind 2 h hyperinsulinemic (9pmol/kg/min) hypoglycemic clamp studies before and after either 6 weeks of fluoxetine (n=8) or identical placebo (n=10). Glucose kinetics were 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. Autonomic nervous system, neuroendocrine and metabolic counterregulatory responses remained unchanged in the placebo group. However, fluoxetine administration significantly (p<0.05) increased key ANS (epinephrine, norepinephrine, MSNA), metabolic (Endogenous glucose production (EGP), lipolysis) and cardiovascular (systolic blood pressure) counterregulatory responses during hypoglycemia.

**Conclusion:** This study has demonstrated that 6 weeks 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.
Selective Serotonin Reuptake Inhibitors (SSRIs) are effective drugs for the treatment of depressive disorders associated with reduced serotonergic function. Serotonergic neurons play an important role in the regulation of neuroendocrine function carried out via both sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) pathways.

Two studies have reported increased hypoglycemia and loss of awareness to hypoglycemia related to the use of SSRIs in depressed patients with type 1 diabetes (1, 2). Although SSRIs are potent inhibitors of neuronal serotonin uptake, they also have the ability to block norepinephrine transport (3, 4). This would be predicted to increase sympathetic outflow activity (4-7). Supporting this, previous studies by a number of investigators have demonstrated that SSRIs can modulate sympathetic nervous system activity and increase counterregulation in rats (8).

Two recent studies in healthy humans (9) and conscious rats (10) have provided further insight into the effects of SSRIs on counterregulatory physiology during hypoglycemia. Briscoe, et al. (9) investigated the effects of 6 weeks high dose fluoxetine administration on physiologic responses to hypoglycemia in a group of healthy, non-depressed humans. Key sympathetic nervous system (epinephrine, norepinephrine, muscle sympathetic nerve activity) and metabolic (glucose production, lipolysis) counterregulatory mechanisms were significantly amplified by the SSRI. Sanders, et al. (10) elegantly studied the chronic effects of another SSRI (sertraline) in a conscious rat model. Following 20 days administration of the SSRI, epinephrine and glucagon responses were significantly increased during hypoglycemia. Additionally, sertraline preserved levels of epinephrine during repeated hypoglycemia, thereby preventing the blunting effects of antecedent hypoglycemia on subsequent ANS counterregulatory responses. Taken together, the above data suggest that serotonergic transmission may be an important mechanism in up-regulating sympathetic nervous system drive during hypoglycemia in both rats and healthy man.

However, the effects of SSRIs on ANS, neuroendocrine and metabolic counterregulatory mechanisms during hypoglycemia in type 1 diabetes do not appear to have been studied. To address this question, the specific aim of this study was to test the hypothesis that chronic administration of the commonly used SSRI, fluoxetine, would result in an amplification of metabolic and ANS counterregulatory mechanisms during hypoglycemia in non-depressed, individuals with T1DM. The glucose clamp technique was used so that insulin and glucose levels could be controlled in all studies.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Twenty T1DM (15M/5F) (age range 19yrs - 48yrs, BMI 25±3 kg/m², diabetes duration 18±9 years and HbA1c 7.0±0.4% (normal range 4-6.5%) were studied. The Zung Self-Rating Depression Scale (11, 12) was completed by each subject to rule out symptoms of clinical depression. None had a history of epilepsy or had a history of any major psychiatric illness. None were taking any psychotropic medication. Each subject had a normal blood count, plasma electrolytes, liver and renal function. All gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board.

**Experimental Design.** 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 4 glucose tests per day and were asked to avoid
hypoglycemia for at least 5 days before a study). On the day prior to 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 (CRC) at 5:00 PM on the evening before an experiment. At this time, two intravenous cannulae were inserted under 1% lidocaine local anesthesia. One cannula 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 so that arterialized blood could be obtained (13). The other cannula 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⁻¹.

**Hypoglycemia Experiments.** After an overnight 10 hr fast at 0 min, a primed (18 μCi) continuous infusion (0.18 μCi·min⁻¹) of HPLC purified [3-³H] glucose (Perkin Elmer Life Sciences, Boston, MA; 11.5 mCi·mmol⁻¹·l⁻¹) 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 (9.0 pmol·kg⁻¹·min⁻¹) infusion of insulin (Human Regular Insulin, Eli Lilly, Indianapolis, IN) was started and continued until 240 min. The rate of fall of glucose was controlled (0.06 mmol·min⁻¹) and the glucose nadir (2.8 mmol·l⁻¹) was achieved using a modification of the glucose clamp technique (14). During the clamp period, plasma glucose was measured every five min and a 20% dextrose infusion was adjusted so that plasma glucose levels were held constant at 2.8 ± 0.1 mmol·l⁻¹ (15). Potassium chloride (20 mmol·l⁻¹) was infused during the clamp to reduce insulin-induced hypokalemia. After completion of the 2 hr test period, the plasma glucose was rapidly restored to euglycemia with 20% dextrose. A second identical hyperinsulinemic-hypoglycemic clamp was performed after 6 weeks of the study medication.

**Study Medication.** Following the initial one-day clamp study, volunteers were given a one 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 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 gender 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 males and females in the placebo and fluoxetine treatment groups.

During the 6-week treatment period, volunteers came to the GCRC once a week for monitoring of compliance and adverse events. Compliance was determined via a pill count and a blood draw to measure serum fluoxetine levels. Two subjects (1M/1F) in the fluoxetine group withdrew from the study due to side effects. The withdrawals occurred early at the 20 mg dose and were described as feelings of tiredness and non-specific 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 hypoglycemic clamp study as previously
described. Thus, 18 subjects (8 fluoxetine/10 placebo) completed both hypoglycemia clamp studies. Upon completion of this second one-day study, subjects were tapered off the study medication (placebo or fluoxetine). Those receiving fluoxetine were given one week at 40 mg/day and one week at 20 mg/day. Once subjects finished the medication, they were unblinded as to the medication they had taken.

**Direct Measurement of Muscle sympathetic Nerve activity (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 1000 Hz channel-1 (DATAQ Instruments Inc. Akron, OH). Five min Windaq files were analyzed with a MatLab GUIDE interface (to adjust for an individual’s 1.3 sec nerve burst delay from a once-removed R-R interval, automatically detected by; pulse synchronicity, a 2:1 signal to noise ratio and wave form shape. Further criteria for acceptable MSNA recordings were: 1) electrical stimulation 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 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 both EGP and any exogenous glucose infused to maintain the desired hypoglycemia) and subtracting it from the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative, since 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, in order 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 hypoglycemic 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 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 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%. Pancreatic polypeptide was measured by RIA using the method of Hagiopian et al. (24) with an interassay CV of 8%. Lactate, glyceral, alanine and β-hydroxybutyrate were measured in deproteinized whole blood using the method of Lloyd et al. (25). Non-esterified fatty acids (NEFA) 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 were 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 non-
invasively 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 experience of the symptoms twice during the control period and every 15 min during experimental periods. Symptoms measured included sweaty, tremor/shaky, hot, thirsty/dry mouth, agitation/irritability, palpitations, tired/fatigued, confusion dizzy difficulty thinking, blurriness of vision, and sleepy. The ratings of the first six symptoms were summed to get the autonomic score while the ratings from the last six symptoms provide a neuroglycopenic symptom score.

**Statistical Analysis.** Data are expressed as mean ± SE and were analyzed using standard, parametric, one- and two-way analysis of variance (ANOVA) and with repeated measures where appropriate (SigmaStat; SPSS Science, Chicago, IL). Tukey’s post hoc analysis was used delineate statistical significance across time within each group, and for each group compared to the PE control group. A P value of <0.05 was accepted as statistically significant. The baseline and final 30 min of hypoglycemia was compared for most parameters, as steady state glucose levels, insulin levels, and glucose infusion rates were achieved by this time.

**RESULTS**

**Glucose, Insulin and Fluoxetine Levels.** Basal plasma glucose levels were 6.3±0.3 and 5.6±0.2 mmol/L in the pre-fluoxetine and pre-placebo clamps. Basal glucose levels were similar (6.1±0.3 and 5.5±0.3 mmol/L) following 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 (Figure 1). Basal and steady state insulin levels for both fluoxetine and placebo groups were similar during both pre- (120±18; 660±24 pmol/L; 126±30; 648±54 pmol/L, respectively) and post- (120±18; 630±42 pmol/L; 144±30; 667±36 pmol/L, respectively) clamp studies (Figure 2). HbA1c levels were unchanged during fluoxetine (7.0±0.3 to 6.8±0.3%) and placebo administration (6.9±0.3 to 6.9±0.3%). Similarly, weight was constant during both fluoxetine (74.2±13 to 73±7±14.3 kg) and placebo administration (77.5±9 to 77.4±9 kg). 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 post-fluoxetine (5436±808 pmol/L) compared to pre-treatment (3815±841 pmol/L) and post-placebo (3198±791 pmol/L) hypoglycemic clamp. Epinephrine responses were similar during the final 30 min of the pre- and post-placebo hypoglycemic clamps (Figure 2).

Norepinephrine responses were significantly higher (p<0.05) during the final 30 min of post-fluoxetine (2.3±0.3 nmol/L) as compared to pre-treatment (1.7±0.2 nmol/L) and post-placebo (1.6±0.2 nmol/L). Norepinephrine responses were similar during the final 30 min of hypoglycemia during the pre- and post-placebo studies (Figure 2).

Cortisol levels were increased (p<0.05) in the post-fluoxetine vs. the post-placebo groups (800±55 vs. 635±83 nmol/L). Cortisol levels remained unchanged during placebo administration.

Peak pancreatic polypeptide levels during hypoglycemia were practically unchanged (135±37 vs. 138±27 nmol/L) after fluoxetine administration as compared to pre-treatment values, respectively. Pancreatic polypeptide levels were also similar for the control group.
post-placebo (115±35 nmol/L) vs. pre-placebo (121±26 nmol/L) during the final 30 min of hypoglycemia (Figure 3).

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

Muscle Sympathetic Nerve Activity. Muscle sympathetic nerve activity increased by a significantly greater amount (p<0.05) during hypoglycemia post-fluoxetine (16±3 bursts/min) vs. both post-placebo (10±2 bursts/min) and pre-fluoxetine (5±2 bursts/min). There were no differences in the post-placebo vs. pre-placebo responses (Figure 4).

Glucose Kinetics. Glucose specific activity (disintegrations per min per mg) was in a steady state during the basal period and the final 30 min of all hyperinsulinemic-hypoglycemic clamps (Table 1).

Endogenous glucose production (EGP) in post-fluoxetine (11±1.1 μmol/kg/min) was significantly increased (p<0.05) during the final 30 min of hypoglycemia as compared to pre-fluoxetine (5.1±1.1 μmol/kg/min), pre-placebo (2.2±1.6 μmol/kg/min) and post-placebo (5.0±2.2 μmol/kg/min). Glucose infusion rates were 14.3±6.1 and 8.9±3.9 μmol/kg/min post-placebo and post-fluoxetine, respectively. Glucose rate of disappearance (Rd) during the final 30 min of hypoglycemia were 19±2.8 and 19.8±3.3 following post-placebo and fluoxetine, respectively (Figure 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) following fluoxetine (115±18μmol/L) as compared to post-placebo (83±16μmol/L) or pre-fluoX (73±5μmol/L). There was no difference in the increase of glyceral in the pre- and post-placebo studies (Table 2).

There was an increase in NEFA levels during hypoglycemia post-fluoxetine (182±64 μmol/L) as compared to post-placebo (91±25μmol/L) and pre-fluoxetine (93±24 μmol/l) (p<0.05) studies. There was no difference in the increase of NEFA levels during hypoglycemia in the pre- and post-placebo studies. 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 6 weeks administration of fluoxetine. Heart rate was significantly higher (p<0.05) during the final 30 min of post-fluoxetine as compared to pre-fluoxetine and post-placebo groups (80±4 vs. 74±5 and 69±5 beats/min; respectively). Heart rate was similar in the control group (69±5 (post-placebo) vs. 67±8 (pre-placebo beats/min)). Systolic blood pressure was significantly increased during hypoglycemia in the post-fluoxetine group vs. pre-fluoxetine and post-placebo groups (127±4 vs. 113±3 and 115±6 mm Hg, respectively; p< 0.05). There were no differences in systolic blood pressure in the placebo control group (115±6 (post-placebo) vs. 119±5 (pre-placebo) mm Hg). Diastolic blood pressure was also increased post-fluoxetine (p<0.05) as compared to post-placebo and pre-fluoxetine (67±2 vs. 61±3 and 61±1 mm Hg, respectively (Table 3).

Symptom Response. There were no differences in total symptom scores in the experimental group when compared to either pre-fluoxetine or post-placebo. Similarly, no significant difference occurred in the post-placebo control group as compared to the pre-
placebo clamp study (Figure 4). Both autonomic and neuroglycopenic symptoms scores were similar during hypoglycemia before and after fluoxetine. (autonomic symptoms pre-fluoxetine was 23±4 vs. 19±4 post-fluoxetine; neuroglycopenic scores were 19±4 pre-fluoxetine and 20±4 post-fluoxetine).

**DISCUSSION**

This study has determined the effects of 6 weeks administration of the selective serotonin reuptake inhibitor fluoxetine on counterregulatory responses to hypoglycemia in non-depressed patients with type 1 diabetes. Our results demonstrate that 6 weeks of high dose fluoxetine significantly increases sympathetic nervous system, hypothalamo-pituitary-adrenal and metabolic (endogenous glucose production, lipolysis) counterregulatory responses in patients with long duration type 1 DM. Hypoglycemia remains the major barrier to even near normalization of glucose in patients with type 1 DM. We have recently reported the beneficial effects of 6 weeks fluoxetine administration on amplifying counterregulatory mechanisms during hypoglycemia in non-diabetic 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 DM. Similar to non-diabetic 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 endogenous glucose production and lipolysis.

In longer duration type 1 DM 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 DM (29). Unfortunately in T1DM 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 non-diabetic 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 DM. Thus the amplified (doubled) EGP response following fluoxetine represents a potentially important defense against hypoglycemia in type 1 DM. 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 non-esterified fatty acids (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 is relatively flat (33, 34) with increasing the concentrations of the catecholamines from 600 to 1000 pg/ml, producing limited actions.
Similar to the findings in healthy man, fluoxetine resulted in an increased response of cortisol during hypoglycemia. This also demonstrates that the SSRI was having effects to activate multiple neural pathways within the CNS (7).

Accompanying the amplified SNS responses were significant increases in blood pressure and heart rate during hypoglycemia following fluoxetine. Somewhat surprisingly, there was not an increase in symptom scores despite the significant increases in central ANS drive and the respective target organ responses (i.e. liver, adipose tissue, heart) following fluoxetine. This apparent dissociation 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 non-diabetic 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 non-diabetic 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 DM rather than simply just a loss of ANS input into the alpha cells (37).

Another point that should be noted is that fluoxetine only amplified counterregulatory responses during hypoglycemia but 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 non-diabetic conscious rats. We also found that chronic administration of the SSRI fluoxetine in non-diabetic individuals had no effects on increasing basal cardiovascular, ANS and metabolic function. However, basal plasma cortisol (but not glucagon and growth hormone) were increased by fluoxetine in non-diabetic 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 has 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 man and conscious rats (9, 10). The findings of increased cortisol and catecholamines can not 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 muscle sympathetic nerve activity was increased in the present study certainly indicates that central sensing and action of the SSRI were occurring. The affects of SSRIs on HPA responses during stress appears to be more complex. We have found that fluoxetine can increase plasma cortisol responses during hypoglycemia in both
healthy and type 1 DM man. Durand, et al. have also demonstrated that fluoxetine can amplify corticosteroid responses to stress in conscious rats (40). However, Sanders, et al., studying a different SSRI (sertraline) and species of rat (Sprague Dawley), found no amplification of HPA axis responses during hypoglycemia (10). Thus, the physiologic effects of SSRI may be different depending upon the specific agent and experimental model under investigation.

The present study has provided an evaluation of fluoxetine’s effects on physiologic responses during hypoglycemia in a group of metabolically well controlled type 1 DM. These individuals typically have the highest prevalence of hypoglycemia and might be expected to benefit most from strategies aimed at improving ANS responses. Six weeks 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 as 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 nor had 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 HbA1c were no different in the placebo or fluoxetine groups. Rates of hypoglycemia (albeit anecdotally reported by the patients) appeared to be either unchanged (n=3), improved (n=3) or relatively improved (n=2) (i.e. unchanged frequency of hypoglycemia with improvement in HbA1c 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 step-wise fashion to 80 mg in an attempt to limit side effect 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 non-specific 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 non-diabetic 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, muscle sympathetic nervous system), metabolic (endogenous glucose production, lipolysis) and cardiovascular counterregulatory response during clamped moderate (2.8 mmol/L) hypoglycemia in type 1 DM. The study also demonstrates that serotonergic mechanisms can play a significant role in regulating ANS and HPA physiologic responses during hypoglycemia in type 1 DM man. In conclusion, these present results have provided novel findings demonstrating, that under the conditions of the present study, SSRIs (specifically fluoxetine) may provide beneficial adjunct effects in amplifying epinephrine levels during hypoglycemia in type 1 DM man.

ACKNOWLEDGEMENTS
The authors would like to thank the expert technical assistance of Eric Allen, Susan Hajizadeh, Nathan Jones and Mary Garmon.
We would also like to acknowledge the superb care provided by the staff of the Vanderbilt General Clinical Research Center. Support for this work was provided by grants from the NIH R01-DK-069803, MO1-RR-00095, P01-HL-056693 and P60-DK-020593.

REFERENCES


Table 1. Glucose specific activity (dpm/mmol) during the basal period and the final 30 min of hyperinsulinemic-hypoglycemic clamps in subjects with T1DM before and after 6 weeks of fluoxetine or placebo.

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Data are mean ± SE.
Table 2. Plasma glycerol, lactate, β-hydroxybutyrate, NEFA and alanine, levels during basal period and final 30 min of hyperinsulinemic hypoglycemic clamp studies in T1DM before and after 6 weeks of fluoxetine or placebo.

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</tr>
<tr>
<td>Pre-treatment</td>
<td>58±7</td>
<td>65±8</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>95±25</td>
<td>110±21*</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>67±15</td>
<td>74±15</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>73±11</td>
<td>80±17</td>
</tr>
<tr>
<td><strong>Lactate (mmol/L)</strong></td>
<td></td>
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</tr>
<tr>
<td>Pre-treatment</td>
<td>0.4±0.04</td>
<td>0.75±0.09</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>0.3±0.02</td>
<td>0.83±0.15</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>0.4±0.04</td>
<td>0.75±0.09</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>0.4±0.05</td>
<td>0.84±0.00</td>
</tr>
<tr>
<td><strong>β-hydroxybutyrate (μmol/L)</strong></td>
<td></td>
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</tr>
<tr>
<td>Pre-treatment</td>
<td>0.099±0.089</td>
<td>0.05±0.03</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>0.046±0.026</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>0.07±0.004</td>
<td>0.019±0.002</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>0.07±0.005</td>
<td>0.02±0.01</td>
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<tr>
<td><strong>NEFA (μmol/L)</strong></td>
<td></td>
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</tr>
<tr>
<td>Pre-treatment</td>
<td>149±38</td>
<td>93±24</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>165±41</td>
<td>182±64 *</td>
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<tr>
<td>Pre-treatment</td>
<td>131±34</td>
<td>86±22</td>
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<tr>
<td>Post-placebo</td>
<td>212±37</td>
<td>91±24</td>
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<td></td>
<td>Pre-treatment</td>
<td>Post-fluoxetine</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>Alanine (μmol/L)</strong></td>
<td>0.23±0.02</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td></td>
<td>0.22±0.02</td>
<td>0.21±0.03</td>
</tr>
</tbody>
</table>

Data are mean ± SE. * represents a significant increased responses during final 30 min of hypoglycemia following 6 weeks of fluoxetine. (*p<0.05).
Table 3. Cardiovascular responses during hyperinsulinemic-hypoglycemic clamp studies in T1DM before and after 6 weeks of fluoxetine or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Basal period</th>
<th>Final 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td></td>
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</tr>
<tr>
<td>Pre-fluoxetine</td>
<td>69±4</td>
<td>74±5</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>69±4</td>
<td>80±4*</td>
</tr>
<tr>
<td>Pre-placebo</td>
<td>67±5</td>
<td>67±8</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>65±5</td>
<td>69±5</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-fluoxetine</td>
<td>107±3</td>
<td>113±3</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>115±7</td>
<td>127±4*</td>
</tr>
<tr>
<td>Pre-placebo</td>
<td>110±3</td>
<td>119±5</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>110±4</td>
<td>115±6</td>
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<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
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<td></td>
</tr>
<tr>
<td>Pre-fluoxetine</td>
<td>66±1</td>
<td>61±1</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>68±3</td>
<td>67±2*</td>
</tr>
<tr>
<td>Pre-placebo</td>
<td>67±3</td>
<td>63±3</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>65±3</td>
<td>61±3</td>
</tr>
</tbody>
</table>

Data are mean ± SE. * represents a significant increased responses during final 30 min of hypoglycemia following 6 weeks of fluoxetine. (*p<0.05).
Figure 1. Plasma glucose and insulin concentrations (mean ± SE) during hypoglycemic clamp studies in 18 (14 men/4 women) with T1DM before and after 6 weeks of fluoxetine or placebo.

Figure 2. Mean plasma epinephrine, norepinephrine and cortisol levels (mean ± SE) during the basal period and the final 30 min of hypoglycemic clamp studies in 18 (14 men/4 women) with T1DM before and after 6 weeks of fluoxetine or placebo. *Plasma epinephrine, norepinephrine and cortisol levels are significantly increased (p<0.05) following fluoxetine.
Figure 3. Mean plasma pancreatic polypeptide, glucagon and growth hormone levels (mean ± SE) during the basal period and the final 30 min of hypoglycemic clamp studies in 18 (14 men/4 women) with T1DM before and after 6 weeks of fluoxetine or placebo.

Figure 4. Delta MSNA and total Symptom Responses during the final 30 min of hypoglycemic clamp studies in 18 (14 men/4 women) with T1DM before and after 6 weeks of fluoxetine or placebo. Data are mean ± SE. *MSNA responses are significantly increased (p<0.05) following fluoxetine.
Figure 5. Glucose kinetics during the basal period and the final 30 min of hypoglycemic clamp studies in 18 (14 men/4 women) with T1DM before and after 6 weeks of fluoxetine or placebo. Data are mean ± SE. *Endogenous glucose production is significantly increased (p<0.05) following fluoxetine.