Effects of a Selective Serotonin Reuptake Inhibitor, Fluoxetine, on Counterregulatory Responses to Hypoglycemia in Healthy Man

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Objective: Hypoglycemia commonly occurs in intensively treated patients with Diabetes. Repeated hypoglycemia blunts counterregulatory responses thereby increasing the risk for further hypoglycemic events. Currently, physiologic approaches to augment counterregulatory responses to hypoglycemia have not been established. Therefore the specific aim of this study was to test the hypothesis that 6 weeks administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine would amplify autonomic nervous system (ANS) and neuroendocrine counterregulatory mechanisms during hypoglycemia.

Research Design and Methods: Twenty healthy (10M/10F) subjects participated in an initial single step hyperinsulinemic (9pmol/kg/min) hypoglycemic (2.9±0.1 mmol/l) clamp study and were then randomized to receive 6 weeks of fluoxetine (n=14) or identical placebo (n=6) in a double blind fashion. After 6 weeks, subjects returned for a second hypoglycemic clamp. Glucose kinetics were determined by 3-tritiated glucose, and muscle sympathetic nerve activity (MSNA) was measured by microneurography.

Results: Despite identical hypoglycemia (2.9±0.1 mmol/l) and insulinemia during all clamp studies, key ANS (epinephrine, norepinephrine, MSNA but not symptoms), neuroendocrine (cortisol) and metabolic (endogenous glucose production, glycogenolysis, lipolysis) were increased (p<0.01) following fluoxetine.

Conclusion: This study has demonstrated that 6 weeks administration of the SSRI fluoxetine can amplify a wide spectrum of ANS and metabolic counterregulatory responses during hypoglycemia in healthy man. These data further suggest that serotonergic transmission may be an important mechanism in modulating sympathetic nervous system (SNS) drive during hypoglycemia in healthy man.
Several reports have indicated that fluoxetine could have metabolic effects and influence carbohydrate metabolism (1-3). In fact, there have been three case studies reporting the occurrence of hypoglycemia related to the use of SSRIs in depressed patients with and without Diabetes (4-6). However, although SSRIs are potent inhibitors of neuronal serotonin uptake, they also have the ability to block norepinephrine transport (7). This would be predicted to increase sympathetic outflow activity (2,8). Supporting this, Baudrie and Chaouloff have previously reported an increased hyperglycemic response to 2-deoxy-d-glycose in conscious rats following serotononergic receptor antagonists implying increased counterregulation in these animals (9).

Subsequent studies by Perry and Fuller demonstrated that systemic injection of the SSRI fluoxetine in rats resulted in 3-fold increases of hypothalamic norepinephrine release (2); thereby providing a mechanistic basis for SSRIs to modulate sympathetic nervous system activity. Later work by Bymaster et al. examined the specificity of five different SSRIs (fluoxetine, citalopram, fluvoxamine, paroxetine and sertraline) to acutely increase the extracellular concentration of serotonin and norepinephrine in rat forebrains. The study demonstrated that among the SSRIs examined; only fluoxetine increased extracellular concentrations of both norepinephrine and serotonin in the rat brains, and suggested that fluoxetine may have differential effects as compared to other SSRIs (3).

Thus, previous information from depressed humans and physiologic data from healthy rats have provided conflicting data concerning possible effects of SSRIs on counterregulatory mechanisms. In addition, despite the widespread clinical use of SSRIs, there have been no clinical studies evaluating the effects of prolonged administration of these agents on counterregulatory responses during clamped hyperinsulinemic hypoglycemia. In the present study, fluoxetine was chosen based on the drug’s frequent use in clinical practice and data demonstrating physiologic effects on both serotonergic and norepinephrine transport in rats. The hypoglycemic clamp technique was used to quantify autonomic nervous system (ANS), neuroendocrine and metabolic counterregulatory mechanisms prospectively before and after 6 weeks administration of fluoxetine in healthy non-depressed man.

**RESEARCH DESIGN AND METHODS**

**Subjects**

Twenty healthy volunteers (10M/10F), 29±2 yrs, (range 20-44 yrs) with a body mass index of 24±3 kg/m², and glycosylated hemoglobin (HbA1c) of 5.3±0.1% (normal range 4-6.5%) were studied. The Zung Self-Rating Depression Scale (10,11) was completed by each subject to rule out symptoms of clinical depression. None had a history of epilepsy or any major psychiatric illness. None were taking any psychotropic medication. Each subject had a normal blood count, plasma electrolytes, liver and renal function. Three subjects had a family history of Diabetes. All gave written informed consent. Hypoglycemia (2.9 mmol/L) and euglycemia (~5.0 mmol/L) studies were approved by the Vanderbilt University Human Subjects Institutional Review Board.

**Experimental Design**

Twenty subjects participated in two separate hypoglycemia studies, separated by at least 6 weeks (see figure 1). Subjects received the study medication (n=14; 7M/7F) or placebo (n=6; 3M/3F) in a randomized, double-blind fashion after completion of their first clamp study.

The subjects were asked to avoid any exercise and consume their usual weight maintaining diet for 3 days before each study. Each
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subject was admitted to the Vanderbilt General Clinical Research Center (GCRC) on the evening before an experiment. All subjects were studied after an overnight 10 hr fast.

On the morning of each study, two intravenous cannulae were inserted under 1% lidocaine local anestheia. One cannula was placed in a retrograde fashion into a vein on the back of the hand. This hand was placed in a heated box (55-60°C) so that arterialized blood could be obtained (12). The other cannula was placed in a large vein in the contralateral arm so that 20% glucose could be infused via a variable rate volumetric infusion pump (Imed San Diego, CA).

Hypoglycemia Experiments

After insertion of venous cannulae, at 0 min, a primed (18μCi) 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 started. At time 120 min, a primed constant (9.0 pmol·kg⁻¹·min⁻¹) infusion of insulin (Eli Lilly, Indianapolis, IN) was started and continued until 240 min. The rate of fall of glucose was controlled (0.06mmol·min⁻¹) and the glucose nadir (2.9mmol·l⁻¹) was achieved using a modification of the glucose clamp technique (13,14). Potassium chloride (20mmol·l⁻¹) was infused during the clamp to reduce insulin-induced hypokalemia. A second identical hyperinsulinemic-hypoglycemic clamp was performed after receiving 6 weeks of study medication.

Study Medication

Following the initial clamp study, volunteers were given 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. Volunteers were blinded as to the treatment group to which they were assigned. Stratified block randomization was performed by the Vanderbilt University Investigational Pharmacy. The subjects were stratified according to sex, because gender is known to affect counterregulatory responses (15). 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. The study was powered at n=14 for the fluoxetine group. When we reached this total, it became obvious that there were clear statistical differences between the groups, and it was not necessary to study additional placebo subjects. The placebo group was used primarily as a time control to demonstrate that counterregulatory responses to hypoglycemia in our normal subjects had not changed during the 6 week study period or that involvement in the experimental protocol did not influence physiologic responses to hypoglycemia.

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. One subject in the placebo group withdrew from the study due to perceived side effects of the treatment. After taking either placebo or fluoxetine for 6 weeks, subjects underwent another single day hypoglycemic clamp study as previously described. Upon completion of this second one-day study, subjects were tapered off the study medication (placebo or fluoxetine). Those randomized to fluoxetine received 40 mg/day for one week and 20 mg/day for a second week. 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 (16,17). The approximate location of this nerve was determined by transdermal electrical stimulation to produce painless muscle contraction of the foot. Following this, a reference stainless steel microelectrode
with a shaft diameter of 200 µm was placed subcutaneously. A similar tungsten electrode, with an uninsulated tip was inserted into the nerve and used for recording of MSNA.

Nerve activity was recorded on a PC based Windaq data acquisition system at 1000 Hz channel (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 one-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. (18). 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 and insulin were measured as previously described (19,20) with an interassay CV of 12% and 9%, respectively. Catecholamines were determined by HPLC (21) with an interassay of 12% for epinephrine and 8% for norepinephrine. Cortisol was assayed using the Clinical Assays Gamma Coat Radioimmunoassay (RIA) kit with an interassay CV of 6%. Growth hormone and pancreatic polypeptide were determined by RIA (22,23) with CVs of 8.6% and 8.0%, respectively. Lactate, glycerol, alanine and β-hydroxybutyrate were measured in deproteinized whole blood using the method of Lloyd et al. (24). Nonesterified fatty acids (NEFA) were measured using the WAKO kit adopted for use on a centrifugal analyzer (25). Hemoglobin A1C was determined in whole blood using the Variant™ II Hemoglobin A1C cation exchange HPLC kit system (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. Hypoglycemic symptoms were quantified using a previously validated questionnaire using the model of Deary, et al (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.
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 control group. A $P$ value of <0.05 was accepted as statistically significant. Baseline hypoglycemic clamp data represent an average of time points (110 and 120 min), and final 30 min data from each clamp represent an average of three measurements taken during (210, 225, 240 min).

Materials
HPLC-purified [3-3H] glucose (New England Nuclear, Boston, MA) was used as the glucose tracer (11.5 mCi/mmol/l). Human regular insulin was purchased from Eli Lilly (Indianapolis, IN).

RESULTS
Glucose, Insulin and Fluoxetine Levels
Basal plasma glucose levels were 5.3±0.2 during the pre fluoxetine study and 5.1±0.06 mmol/L during the post-fluoxetine study. Weight was unchanged during both the 6 week fluoxetine (71.1±3.3 to 70.0±3.2 kg) and placebo (74.5±6.1 to 74.8±5.4 kg) studies. Plasma glucose levels reached steady state by 30 min and identical hypoglycemia was maintained with plasma glucose levels of 2.9±0.05 mmol/l during clamp procedures for all study groups (see figure 2). Basal and steady state insulin levels for both fluoxetine and placebo groups were similar during both pre- (43±1; 631±5 pmol/l) and post- (37±1; 567±5 pmol/l; 35±2; 559±17 pmol/l, respectively) clamp studies (see figure 2). Mean fluoxetine and norfluoxetine levels at the end of the study were 336.7±61 and 230±41 ng/ml, respectively, in the SSRI group and were undetectable in the placebo group.

Neuroendocrine Counterregulatory Hormones
Epinephrine responses were significantly higher (8187±1365 pmol/l; $p<0.001$) during the final 30 min of hypoglycemia post-fluoxetine compared to pre-treatment (5065±797 pmol/L) and following placebo (4366±508 pmol/l). Epinephrine responses were similar (4366±508 vs. 5065±797 pmol/l) during the final 30 min post-placebo compared to pre-treatment hypoglycemic clamps (see figure 3).

Norepinephrine responses were significantly higher (2.4±0.2 nmol/l) during the final 30 min of post-fluoxetine as compared to the pre-treatment (1.8±0.2 nmol/l) and placebo (1.2±0.1 nmol/l) ($p<0.01$) hypoglycemic clamps. Norepinephrine responses during hypoglycemia following placebo were similar to pre-treatment values, and significantly reduced compared to the fluoxetine group (see figure 3).

Basal cortisol levels were significantly increased (p<0.05) in the fluoxetine group (552±55 nmol/l) as compared to pre-treatment and placebo (359±27 and 304±55 nmol/l). Plasma cortisol responses were also significantly higher (1242±110 and 883±55 nmol/l; $p<0.01$) during the final 30 min of post-fluoxetine vs. pre-treatment and following placebo (678±79 nmol/l). However, no significant differences occurred in the placebo group (see figure 3).

Peak pancreatic polypeptide levels during hypoglycemia increased to 214±54 vs. 149±22 nmol/l; $p<0.06$) after fluoxetine administration as compared to pre-treatment values respectively. Pancreatic polypeptide levels during the final 30 min of hypoglycemia post-fluoxetine were significantly increased compared to post-placebo (165±19 vs. 122±17 nmol/l; $p<0.01$).

Glucagon responses were similar during hypoglycemia in all groups (post-fluoxetine or post-placebo) (see figure 3). Fluoxetine
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had no effect on growth hormone responses during hypoglycemia. Growth hormone increased from 2±1 to 28±6 ng/l pre-treatment; 2±1 to 29±7 ng/l post-treatment, and 2.5±1 to 21±7 ng/l during placebo.

Glucose Kinetics
Glucose specific activity (disintegrations per minute per mg) was in a steady state during the basal period and the final 30 min of all hyperinsulinemic-hypoglycemic clamps (table 1). During the final 30 min of hypoglycemia, exogenous glucose infusion rates were significantly less in the fluoxetine group (post-treatment 1.1±0.5 μmol/kg/min vs. both pre-fluoxetine 3.1±1.1 μmol/kg/min, and post-placebo 5.5±1.7 μmol/kg/min; p<0.01). For the placebo group exogenous glucose infusion rates were not significantly changed (pre-treatment 3.3±1.1 vs. post-placebo 5.5±1.7 μmol/kg/min). The endogenous glucose production (EGP) response in post-fluoxetine (14±1.1 μmol/kg/min) was significantly increased (p<0.01) as compared to both pre-treatment levels (10.3±1.1 μmol/kg/min) and post-placebo (9±1.1 μmol/kg/min). EGP in the post-placebo group was similar to pre-treatment 9±1.1 vs. 10.3±1.1 μmol/kg/min). The glucose rate of disappearance (Rd) during the final 30 min of hypoglycemia was not significantly changed as a result of receiving 6 weeks of fluoxetine (see figure 4).

Muscle Sympathetic Nerve Activity
Muscle sympathetic nerve activity increased by a significantly greater amount (p<0.05) during hypoglycemia in the post fluoxetine group (19±3 bursts/min) as compared to both pre-treatment (13±3 bursts/min) and post-placebo (14±3 bursts/min). There were no differences in the post-placebo vs. pre-treatment groups (14±3 bursts/min vs. 13±3 bursts/min, respectively) (see figure 5).

Intermediary Metabolism
Baseline glycerol, lactate, β-hydroxybutyrate, nonesterified fatty acids (NEFA), and alanine levels were similar among groups (Table 2). The increase in glycerol during hypoglycemia (19±4 μmol/l) was significantly greater (p<0.05) following fluoxetine as compared to pre-treatment (12±3 μmol/l) or post-placebo (10±2 μmol/l). There was no difference in the increase of glycerol during hypoglycemia in the placebo group.

Blood lactate was significantly increased during the final 30 min of hypoglycemia in the post-fluoxetine group vs. pre-treatment and placebo groups (1.8±0.2 vs. 1.3±0.08 and 1.1±0.1 mmol/l, respectively; p< 0.05). No significant changes occurred in the control group (post-placebo group vs. pre-treatment (1.1±0.09 vs. 1.3±0.08 mmol/l).

β-hydroxybutyrate levels were also significantly higher in the post-fluoxetine group vs. pre-treatment and placebo groups (0.27±0.01 vs. 0.015±0.01 and 0.017±0.01 mmol/l, respectively; p< 0.05). No differences occurred in the pre and post-placebo group (0.015±0.01 vs. 0.017±0.01 mmol/l, respectively). There was a trend for a greater reduction in NEFA levels during pre-treatment (276± 40 as compared to post-fluoxetine 192±25 (P=0.069).

Cardiovascular Parameters
Basal heart rate and blood pressure were not different after 6 weeks administration of fluoxetine. Heart rate was significantly higher during the final 30 min of post-fluoxetine as compared to pre-treatment and placebo groups (79±6 vs. 69±3 and 70±5 beats/min, respectively; p< 0.05). No significant differences in heart rate were noted for control group (70±5 (post-placebo) vs. 69±3 (pre-treatment beats/min)). Systolic blood pressure was also significantly increased during hypoglycemia in the post-fluoxetine group vs. pre-treatment and placebo groups (129±6 vs. 121±5 and 118±6 mm Hg, respectively; p< 0.05). There were no differences in the increase of systolic blood pressure in the placebo group (118±6 vs. 121±5 mm Hg). No significant changes in
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diastolic blood pressure occurred in experimental or placebo groups (Table 3).

Symptom Response
There were no differences in total symptom scores in the fluoxetine group when compared to either pre-treatment or control groups. (Autonomic symptoms pre-treatment were 10±2 vs. 8±1 post-treatment. Neuroglycopenic scores were 14±3 pre-treatment and 11±1 post-treatment). Symptoms increased similarly in the post-placebo control group when compared to the pre-treatment clamp study (Figure 5).

DISCUSSION
This study investigated hypoglycemic counterregulatory responses following 6 weeks chronic administration of fluoxetine in non-diabetic, non-depressed individuals. We determined that plasma concentrations of key neuroendocrine, ANS and metabolic counterregulatory responses were increased during the final 30 min of clamped moderate hypoglycemia following high dose fluoxetine administration. Key counterregulatory mechanisms (ANS, hypothalamic pituitary adrenal, endogenous glucose production, glycogenolysis and lipolysis) were significantly amplified following fluoxetine.

Six weeks administration of fluoxetine at a stepped dose to 80 mg/day resulted in a substantial increase in most, but not all, ANS responses to hypoglycemia. Plasma epinephrine, norepinephrine, pancreatic polypeptide and MSNA were increased by 25-50% following fluoxetine. ANS drive was only increased during hypoglycemia but not during basal conditions. This indicates that the SSRI did not produce a chronic over stimulation of the sympathetic nervous system, (particularly evidenced by no increase in basal heart rate, systolic blood pressure, norepinephrine and MSNA levels). The fluoxetine increased sympathetic nervous system drive during hypoglycemia therefore appears to be due to an amplification of usual physiologic responses rather than modulation of basal homeostatic mechanisms. Sympathoadrenal, sympathetic neural and MSNA response, were all amplified following fluoxetine. This was distinct to fluoxetine’s effects on plasma cortisol where there was an increase in both basal levels and responses of the hormone during hypoglycemia. The increased sympathetic nervous system drive resulted in significant amplification of metabolic homeostatic mechanisms during hypoglycemia. Of note, glucose kinetics were profoundly influenced by fluoxetine administration. In particular, endogenous glucose production was strikingly elevated by fluoxetine. During prolonged hypoglycemia the ability to defend against a reduced glucose level depends upon the balance of increasing glucose production and limiting glucose utilization. Typically during hypoglycemia that occurs in patients with Type 1 DM, it is the restriction of glucose uptake that is the major homeostatic mechanism as there is little or no endogenous glucose production in these individuals. Fluoxetine administration resulted in a small reduction in glucose uptake but a significant amplification of endogenous glucose production. This latter response may have been due to the combination of the elevated basal cortisol and amplified catecholamines during hypoglycemia (29). Key metabolic counterregulatory mechanisms such as lipolysis (increased glycerol responses), glycogenolysis (increased lactate) and ketogenesis were all elevated by the amplified sympathetic nervous system response caused by fluoxetine. The major regulation of glucose kinetics during hypoglycemia in long standing Type 1 DM and insulin deficient Type 2 DM patients revolves around a functioning sympathetic nervous system. Glucagon responses to hypoglycemia in Type 1 DM are typically lost after only 5 years duration and are also significantly reduced in long standing Type 2 DM (30). Thus the sympathetic nervous
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system and epinephrine becomes the principle defense against a falling plasma glucose level. In the present study, epinephrine responses were increased by chronic fluoxetine therapy whereas the placebo control had no effect on responses of the catecholamine during hypoglycemia. Norepinephrine and MSNA responses during hypoglycemia were also similarly increased following fluoxetine; thus indicating a widespread amplification of the sympathetic neural and sympatho-adrenal responses. Of particular note, however, were the intriguing findings regarding the lack of an increase in autonomic symptoms despite very large increases in sympathetic nervous system activity following fluoxetine.

The origin of hypoglycemic symptoms during hypoglycemia is complex. Previous work had demonstrated scenarios whereby discordant responses between autonomic symptoms and catecholamine responses can exist during hypoglycemia (17,31-33). Generally, studies have determined that autonomic symptoms can be preserved despite reduced activity in other branches of the sympathetic nervous system. For example, DeRosa, et al. (31) have demonstrated that symptom responses are preserved in adrenalectomized subjects whom have no measurable epinephrine levels during hypoglycemia. Additionally, Aftab-Guy, et al. (34,35) have also demonstrated that high levels of plasma epinephrine mimicking values observed during moderate hypoglycemia only produces minor increases in hypoglycemic symptoms. These studies are examples from a large body of work that demonstrate that hypoglycemic symptoms are generated primarily from central ANS drive and end organ responses. Secondly, there are data indicating that hypoglycemic symptoms can be influenced independently from other components of the ANS during hypoglycemia. Dagogo-Jack, et al. (32) have demonstrated that hypoglycemic symptoms can increase before adreno medullary or sympathetic neural responses in patients with hypoglycemia-associated autonomic failure (32). Sandoval, et al. (33) and Davis, et al. (17) have also demonstrated that hypoglycemic symptoms are preserved relative to blunting of other ANS responses following antecedent stress. Thus, previous work would indicate that hypoglycemic symptoms are resultant on central ANS drive and are preserved high in the hierarchy of ANS responses to hypoglycemia. Our finding, therefore, of a relatively reduced symptom response (~20%) following fluoxetine in the context of a generalized increased (~50-60%) ANS drive (MSNA, epinephrine, norepinephrine and pancreatic polypeptide) is interesting and unexpected. This may indicate a role for serotonergic pathways in the generation of symptoms during hypoglycemia in healthy man.

The neural mechanisms responsible for fluoxetine's effects on amplifying counterregulatory responses to hypoglycemia are not evident from this study. Numerous studies have demonstrated interactions between serotonergic (both 5-HT1A and 5-HT3) and catecholamine neuro transmission pathways in multiple areas of the brain (8). These include forebrain (thalamus, hypothalamus) and hind brain nuclei that are known to play important roles in regulating ANS responses during hypoglycemia. Carvalho, et al. (36) have demonstrated that third ventricle injections of fluoxetine in wistar rats resulted in hyperglycemia without accompanying hyperinsulinemia. Pretreatment with a selective CRH antagonist prevented the increase in hyperglycemia. These data support a role for fluoxetine to increase CRH levels which can modulate metabolism via increases in sympathetic nervous system outflow (i.e. hyperglycemia independent of hyperinsulinemia which was suppressed by elevated sympathetic nervous system activity (32)). Earlier work by Chaouloff, et al. (1992) also demonstrated a
role in central serotonergic receptors in the regulation of adrenal catecholamine release (37). Subsequently, Durand, et al. have reported that repeated fluoxetine administration can result in increased adrenal weight and amplified corticosteroid responses to stress in certain strains of conscious rats (38). Thus the above studies document a role for the interaction of central serotonergic receptors and 1) hypothalamo-pituitary-adrenal axis and 2) sympathetic outflow in rats which may provide a mechanistic basis for the novel findings of the present study in humans. It should be noted that fluoxetine had little or no effects on amplifying glucagon responses during hypoglycemia. This has some relevance as glucagon is an important component of the normal counterregulatory response to falling plasma glucose and secondly, there are data demonstrating that the ANS can regulate glucagon release during hypoglycemia (39).

The present study has provided a “proof of principle” in evaluating the integrated physiologic effects of the SSRI fluoxetine on counterregulatory responses during hypoglycemia. Despite the findings of significant amplification of ANS and metabolic counterregulatory responses following fluoxetine, we should note some particular aspects of the study design. The dose of fluoxetine used in this study was increased to 80 mg over a 6 week period and is larger than often used in clinical practice. Despite this, the drug was well tolerated with ~ 33% of subjects reporting a transient reduction of appetite and mild nausea; however, weight was unchanged from start to end of study. One other subject receiving fluoxetine experienced some sexual dysfunction and one subject on placebo reported vivid dreams. Side effects had abated by the final 2 weeks of fluoxetine administration. Additionally, the healthy volunteers recruited into this study were screened to have no depression or depressive symptoms. Furthermore, fluoxetine had no effects on amplifying hypoglycemic symptoms during hypoglycemia.

In summary, this study has demonstrated that 6 weeks administration of fluoxetine can profoundly increase key autonomic nervous system (epinephrine, norepinephrine, pancreatic polypeptide, muscle sympathetic nerve activity), metabolic (endogenous glucose production, lipolysis, glycogenolysis), and cardiovascular counterregulatory responses during clamped moderate (2.9 mmol/l) hypoglycemia. This study also demonstrates the importance of serotonergic mechanisms in regulating ANS and HPA physiologic responses during hypoglycemia in healthy man. In conclusion, these present results have provided novel findings demonstrating that serotonergic transmission may be an important mechanism in modulating ANS drive during hypoglycemia in healthy man.

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REFERENCES


26. Bio-Rad Laboratories, Inc. Hercules, CA. Hemoglobin A$_{1C}$ was determined in whole blood using the Variant™ II Hemoglobin A$_{1C}$ cation exchange HPLC kit system. *ref 270-2101.*


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

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre-treatment</th>
<th>Post-fluoxetine</th>
<th>Post-placebo</th>
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<tr>
<td></td>
<td>-20 min</td>
<td>-10 min</td>
<td>0 min</td>
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<tr>
<td></td>
<td>432±21</td>
<td>428±22</td>
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<td></td>
<td>408±36</td>
<td>415±38</td>
<td>401±38</td>
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</table>

Data are mean ± SD.
Table 2. Plasma glycerol, lactate, β-hydroxybutyrate, NEFA and alanine, levels during basal period and final 30 min hyperinsulinemic/hypoglycemic clamp studies in non-diabetics 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>Glycerol (mmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>50±3</td>
<td>62±6</td>
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<tr>
<td>Post-fluoxetine</td>
<td>64±9</td>
<td>82±11*</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>90±21</td>
<td>100±23</td>
</tr>
</tbody>
</table>

| **Lactate (mmol/l)** |              |              |
| Pre-treatment       | 0.5±0.07     | 1.3±0.08     |
| Post-fluoxetine     | 0.6±0.09     | 1.8±0.2*     |
| Post-placebo        | 0.5±0.13     | 1.1±0.09     |

| **β-hydroxybutyrate (μmol/l)** |              |              |
| Pre-treatment          | 0.03±0.01    | 0.015±0.01   |
| Post-fluoxetine        | 0.06±0.02    | 0.027±0.01*  |
| Post-placebo           | 0.095±0.04   | 0.017±0.01   |

| **NEFA (μmol/l)** |              |              |
| Pre-treatment      | 390±53       | 114±13       |
| Post-fluoxetine    | 332±45       | 141±22       |
| Post-placebo       | 308±87       | 95±20        |

| **Alanine (μmol/l)** |              |              |
| Pre-treatment       | 0.22±0.03    | 0.21±0.01    |
| Post-fluoxetine     | 0.24±0.02    | 0.24±0.02    |
| Post-placebo        | 0.26±0.05    | 0.24±0.03    |

Data are mean ± SE. * represents a significant increased response during final 30 min of hypoglycemia following 6 weeks of fluoxetine. (*p<0.05).
Table 3. Cardiovascular responses during hyperinsulinemic-hypoglycemic clamp studies in non-diabetics 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>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>62±3</td>
<td>69±3</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>62±4</td>
<td>79±6*</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>59±4</td>
<td>70±5</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>116±3</td>
<td>121±5</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>115±4</td>
<td>129±6*</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>111±6</td>
<td>118±6</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>67±2</td>
<td>61±2</td>
</tr>
<tr>
<td>Post-fluoxetine</td>
<td>69±2</td>
<td>65±2</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>63±2</td>
<td>59±4</td>
</tr>
</tbody>
</table>

Data are mean ± SE. * represents a significantly increased response during final 30 min of hypoglycemia following 6 weeks of fluoxetine (*p<0.05).
**Experimental Protocol**

**Protocol #1**

![Experimental Protocol Diagram](image)

- **3-\(^3\)H glucose**
  - Insulin 9 pmol/kg/min
  - Plasma glucose 2.9 mmol/L

**MICRONEUROGRAPHY**

Randomized to 6 weeks of:

- Placebo
- Fluoxetine

<table>
<thead>
<tr>
<th>Week</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 mg</td>
</tr>
<tr>
<td>2</td>
<td>40 mg</td>
</tr>
<tr>
<td>3</td>
<td>60 mg</td>
</tr>
<tr>
<td>4-6</td>
<td>80 mg</td>
</tr>
</tbody>
</table>

**Protocol #2**

After 6 weeks treatment

- **3-\(^3\)H glucose**
  - Insulin 9 pmol/kg/min
  - Plasma glucose 2.9 mmol/L

**MICRONEUROGRAPHY**

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**Figure 1**
Schematic Diagram of Experimental Protocol
Figure 2
Plasma glucose and insulin concentrations (mean ± SE) during hypoglycemic clamp studies in 20 (10 men/10 women) non-diabetic patients before and after 6 weeks of fluoxetine (SSRI) or placebo.
Figure 3
Plasma epinephrine and norepinephrine levels (mean ± SE) during hyperinsulinemic hypoglycemic (2.9±0.1 mmol/l) clamp studies in 20 (10 men/ 10 women) before and after 6 weeks of fluoxetine (SSRI) or placebo. Plasma epinephrine and norepinephrine levels are significantly increased (p<0.01) following fluoxetine as compared to pre-treatment and placebo values.

Plasma glucagon and cortisol levels (mean ± SE) during hyperinsulinemic hypoglycemic (2.9±0.1 mmol/l) clamp studies in 20 (10 men/ 10 women) before and after 6 weeks of fluoxetine (SSRI) or placebo. Plasma cortisol levels are significantly increased (p<0.01) following fluoxetine administration.
**Figure 4**
Glucose kinetics during the basal period and the final 30 min of hyperinsulinemic hypoglycemic (2.9±0.1 mmol/l) clamp studies in 20 (10 men/10 women) before and after 6 weeks of fluoxetine (SSRI) or placebo. Endogenous glucose production is increased and glucose infusion rates are reduced (p<0.01) following fluoxetine administration. Data are mean ± SE.
Figure 5
ΔMSNA during the final 30 min of hyperinsulinemic hypoglycemic (2.9±0.1 mmol/l) clamp studies in 20 (10 men/10 women) before and after 6 weeks of fluoxetine (SSRI) or placebo. ΔMSNA levels are significantly increased (p<0.05) following 6 weeks fluoxetine administration. Data are mean ± SE.

Total symptom scores during the final 30 min of hypoglycemic clamp studies in 20 (10 men/10 women) before and after 6 weeks of fluoxetine (SSRI) or placebo. Data are mean ± SE.