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Activation of Blood Coagulation In Patients with Major Depressive Disorder During Euglycemic Hyperinsulinemia

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Because hyperinsulinemia and major depressive disorder (MDD) are both associated with an increased risk of cardiovascular mortality, we hypothesized that experimental hyperinsulinemia would activate coagulation, particularly in patients with MDD [1–5]. To test this hypothesis we measured markers of coagulation activation and fibrinolytic potential in MDD patients and in controls during euglycemic insulinemia [6]. Our observations are reported here.

MATERIALS AND METHODS

Experimental Subjects

This study was conducted under a protocol approved by the Institutional Review Board of the National Institute of Mental Health. Study subjects were required to be between the ages of 18 and 65 years, with or without depression, to have no significant underlying illnesses (eg., diabetes mellitus, cardiovascular disease), and to be taking no medications, including antidepressants. The diagnosis of depression was based upon the criteria of the American Psychiatric Association (DSM-IV) [7]. Subjects were screened with a complete history and physical examination as well as routine laboratory tests (complete blood counts, serum chemistries, thyroid function tests, lipid profile, pregnancy test). All gave signed, informed consent to participate.

Experimental Design

Subjects were admitted to the Warren G. Magnuson Clinical Center the night before they were studied and remained fasting after 10 PM (except for water). On the morning of the study a 20 gauge intravenous cannula was placed in one arm and connected to a four-way parallel port for the administration of glucose (20% dextrose in water), insulin (400 mU/ml), potassium phosphate (0.7 mEq/ml), and normal saline. A 20 gauge intravenous cannula was placed in the opposite arm for blood sampling. Samples for bedside glucose monitoring were collected every 5 minutes and the glucose infusion adjusted to maintain a plasma level of ~90 mg/dl. Samples

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were taken for insulin and potassium monitoring every 30 minutes. Samples for the laboratory assays described below were obtained immediately prior to the start of the insulin infusion, again at the end of the infusion (2–3 hours later), and 2 hours after the infusion had been stopped.

Laboratory assays

Blood samples for measurement of thrombin-antithrombin (TAT) complexes and factor VIII (fVIII) antigen were drawn into BD Vacutainers™ (BD Vacutainer Systems, Franklin Lakes, NJ) containing 0.11 mol/L citric acid, 15 mmol/L theophylline, 3.7 mmol/L adenosine, and 0.2 mmol/L dipyridamole, pH 5.0, supplied by Diagnostica Stago (Parsippany, NJ). The plasma from these samples was passed through 0.45 µm filters before testing. Blood for the functional fVIII assays was anticoagulated with 0.32% sodium citrate (final concentration) in BD Vacutainer tubes. Samples for plasminogen activator inhibitor-1 (PAI-1) activity and tissue plasminogen activator (tPA) activity were anticoagulated with 0.05 mol/L sodium citrate, pH 5.9 (final) in Stabilyte™ tubes from Biopool (Umea, Sweden).

Commercially available ELISAs were used to measure TAT (Dade Behring, Marburg, Germany), functional PAI-1 and tPA (Biopool), and fVIII antigen (Cederlane Laboratories, Ltd., Ontario, Canada). FVIII activity was quantitated using a STA COAG instrument manufactured by Diagnostica Stago.

Statistical Methods

Demographic data were analyzed by unpaired t-tests or Fisher's exact test. Because measurements of some of the analytes (particularly PAI-1 and TAT) were skewed, log transformations of the laboratory data were performed. Differences in the means of the transformed data were analyzed by factorial or repeated measures ANOVA, as appropriate, with post-hoc comparisons by the Bonferroni/Dunn method with $\alpha = 0.05$, using StatView (SAS Institute, Inc., Cary, NC).

RESULTS

Subjects

Data were collected from 32 subjects with MDD and 18 controls. Demographic information is provided in Table 1. The mean age of the MDD group was significantly greater (42 ± 8.9 years) than the mean age of the control group (34 ± 9.8 years) ($P = 0.007$). Differences in the male-female ratio, body mass index (kg/m^2), and mean steady-state insulin levels (munit/L) did not differ significantly between the groups.

Blood Sample Collections

Because of the numerous blood draws (every 5 minutes for glucose for 2 – 3 hours) venous access failed in a few patients before samples could be collected at all 3 designated time points (immediately before the insulin infusion, at the end of the infusion, and 2 hours after the infusion) for the special parameters measured in this study. Any plasma sample with evidence of hemolysis was not used for testing. Therefore, only 14 – 17 of the 18 controls and 22 – 28 of the 32 MDD patients had adequate plasma samples for testing TAT, fVIII activity, PAI-1, and tPA at all 3 time points.

Thrombin-Antithrombin (TAT) Complexes

Seventeen control subjects and 26 MDD subjects had measurements of TAT at all 3 time points. Pre-treatment mean log TAT in controls and patients did not differ significantly ($P = 0.78$). By repeated measures ANOVA there were no significant differences in mean log TAT among the 3 time points for the controls ($P \geq 0.58$) (Fig. 1). However, there were significant changes in

log TAT for the MDD patients. By post hoc analysis log TAT rose significantly from the pre-insulin level to the end-of-insulin level ($P = 0.01$) and remained significantly elevated 2 hours after the infusion ($P = 0.01$).

Factor VIII

FVIII activity was measured at all 3 time points in 14 controls and 28 subjects with MDD. By repeated measures ANOVA and post hoc analysis of the control data there had been a non-significant decrease in the log of fVIII activity by the end of the insulin infusion ($P = 0.06$) with a subsequent return to baseline ($P = 0.002$) (Fig. 2). In contrast, the log of fVIII activity for the MDD subjects had increased significantly by the end of the insulin infusion ($P = 0.0012$) and then returned to baseline ($P < 0.0001$) (Fig. 2). Whereas the mean log of fVIII activity of the controls and patients did not differ significantly before the insulin infusion began ($P = 0.16$) or 2 hours after it was completed ($P = 0.90$), the mean log of fVIII activity at the end of the infusion was significantly higher in the MDD group than in the controls ($P = 0.0005$). Because of the significant difference in mean age between the control and MDD groups, analysis of covariance was performed to see whether this factor accounted for the observed difference in fVIII activity. However, there was no evidence of an interaction effect ($P = 0.58$).

FVIII antigen measurements were available for only 5 control subjects and 12 with MDD. No significant differences were apparent among the time points in either group of subjects, but the power of the analyses was low (0.111 for controls, 0.347 for MDD patients). Comparing all of the antigen measurements of the controls with all of the measurements for the depressed subjects also failed to reveal any significant difference, but the power of the analysis was only 0.215. However, when the ratio of fVIII activity to fVIII antigen was calculated and the data from all 3 time points for the controls were pooled and compared with the pooled data from all 3 time points for the MDD patients, the power of the analysis was greatly increased (0.80) and the mean ratio for the MDD patients was significantly greater than for the controls ($P = 0.007$).

PAI-1 and tPA Activity

Sixteen controls and 22 patients with MDD had measurements of PAI-1 and tPA activity at all 3 time points. By repeated measures ANOVA the log of PAI-1 activity changed similarly in the controls and the patients. In both there was a significant reduction from before the infusion until the end, but then the logs rose again to approach their initial values. The log of tPA activity did not change significantly over time in either group of subjects.

DISCUSSION

Our data indicate that patients with MDD, compared to controls, show evidence of coagulation activation during euglycemic hyperinsulinemia. After the stress of a 2 – 3 hour insulin infusion, the log concentration of TAT complexes and the log fVIII activity rose significantly in the depressed subjects, but not in the controls, and subsequently returned toward baseline (Fig. 1,2). We measured fVIII antigen to determine whether rapid secretion of the factor, which is a known acute phase reactant, accounted for the change in activity. Although we found no significant differences in antigen over time or between the control and depressed groups, the analyses were hampered by limited power. However, combining the ratios of fVIII activity to antigen from all 3 time points revealed that the mean ratio for the MDD subjects was significantly greater than that for the controls. This suggests that fVIII was more activated in the depressed patients. The same phenomenon has been reported in diabetic children [8]. It is possible that the rise in fVIII activity at the end of the insulin infusions resulted from the activation of fVIII by thrombin [9].

The import of our study is limited by the relatively small number of subjects. We cannot rule out that differences between the two groups of subjects were related to the differences in their age or sex distribution. We also made no attempt to control for variables such as smoking habits. Nevertheless, the differential effect could represent a difference in a specific cellular response to hyperinsulinemia. Many cells have insulin receptors, including platelets and endothelium [10,11]. It is also possible that the physical and psychological stress of the clamp procedure itself affected the MDD patients adversely since mental stress has been shown to elevate TAT and fVIII activity, probably mediated by increased catecholamines [12–17].

We also assessed the effect of euglycemic hyperinsulinemia on fibrinolytic potential by measuring the activities of tPA and PAI-1, but no differences between patients and controls were identified. In both groups log PAI-1 activity fell by the end of the infusion, but log tPA did not change.

Deciphering the mechanisms leading to increased thrombin production in the MDD patients was beyond the scope of our study. Nevertheless, our observations suggest that such studies are warranted.

SUMMARY

In the MDD patients but not the control group the logs of TAT and fVIII activity rose significantly during the period of experimental euglycemic hyperinsulinemia. The change in fVIII activity was not associated with a parallel change in fVIII antigen, suggesting that fVIII had become activated by thrombin. The logs of tPA and PAI-1 activity did not change significantly during the hyperinsulinemia in either group of subjects. The mechanism(s) underlying these observations is not clear.

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ABBREVIATIONS

MDD, major depressive disorder; TAT, thrombin-antithrombin complexes; fVIII, factor VIII; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1.

Figure 1.

Box plots of log TAT concentration for the controls (upper panel) and the depressed subjects (lower panel) before starting insulin, at the end of the infusions, and 2 hours after the infusions were completed. The shaded boxes encompass the central 50% of each data set (the interquartile distance) with the medians represented by horizontal lines. The individual points shown are outliers (more than 1.5 times the interquartile distance from the boxes), while the vertical lines indicate the ranges of the remaining data. The horizontal dotted lines with asterisks show the statistically significant differences ($\alpha = 0.05$) between the data sets based upon repeated measures ANOVA with post hoc comparisons by the Bonferroni/Dunn method.

Figure 2.
Box plots of log fVIII activity. The symbols are the same as for Figure 1.

TABLE

Demographics of study subjects

	Controls	MDD Subjects	P
Number	18	32	
Male/Female	7/11	6/26	0.18
Age (years)	34 ± 9.8	42 ± 8.9	0.007
BMI (kg/m ²)	24 ± 2.7	24 ± 3.6	0.41
Mean insulin during clamp (munits/L)	87 ± 21	83 ± 18	0.55