Objective: The primary objective of this study was to assess whether pentagastrin-induced panic symptoms are associated with release of free fatty acids (FFAs) in a manner that could explain the mechanism of correlations observed between serum cholesterol levels and frequency and severity of panic attacks in patients with panic disorder (PD). A secondary objective was to assess whether pretreatment with ethinyl estradiol (EE) attenuates pentagastrin-induced release of FFAs. Methods: A double-blind, crossover, placebo-controlled study was conducted in which patients with PD and healthy volunteers received 2 injections of pentagastrin, 7–10 days apart, with randomization of the order of pretreatment with placebo and EE. Results: We found a statistically significant, time-dependent release of FFAs in response to pentagastrin challenge. However, this release of FFAs was not attenuated by pretreatment with EE. Conclusions: These results support the hypothesis that release of FFAs in association with panic attacks occurs in a manner similar to the stress-induced lipolysis model. This suggests a possible mechanism for the elevated serum cholesterol levels observed in patients with PD. However, the occurrence of a delayed increase in low-density lipoprotein (LDL) cholesterol following induction of a panic attack remains to be tested in studies incorporating a placebo injection visit and timed measurements of LDL cholesterol.

Objectif : Cette étude visait principalement à déterminer si les symptômes de panique produits par la pentagastrine sont associés à la libération d’acides gras libres (AGL) d’une façon qui pourrait expliquer le mécanisme des liens observés entre les taux de cholestérol sérique et la fréquence et la gravité des crises de panique chez les patients qui ont un trouble panique (TP). L’étude visait aussi à évaluer si un traitement préalable à l’éthinyloestradiol (EE) atténue la libération d’AGL provoquée par la pentagastrine. Méthodes : On a procédé à une étude contrôlée par placebo, croisée et à double insu au cours de laquelle des patients qui avaient un TP et des bénévoles en bonne santé ont reçu deux injections de pentagastrine séparées par un intervalle de 7 à 10 jours et l’ordre du traitement préalable au placebo et à l’EE a été randomisé. Résultats : Nous avons constaté une libération chronologique significative sur le plan statistique d’AGL en réponse à la provocation par la pentagastrine. Le traitement préalable à l’EE n’a toutefois pas atténué cette libération d’AGL. Conclusions : Ces résul-

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Introduction

Cardiovascular disease is the most frequent cause of death in developed nations. Although the traditional study of cardiovascular disease has explored the role of conventional risk factors, more recently, greater interest has focused on the association with psychiatric illness, in particular, panic disorder (PD). PD is a common anxiety disorder afflicting 2%–5% of the general population, the hallmark of which is the repeated occurrence of panic attacks (PAs). These PAs are accompanied by psychological symptoms (such as anxiety or fear of dying, of losing control or of “going crazy”), as well as dramatic physical symptoms (such as chest pain, heart palpitations, shortness of breath and hot flashes).

Cardiovascular risk is reportedly higher in patients with PD than in the general population. For example, in 2 retrospective studies, Coryell et al reported that the expected rate of death from cardiovascular disease was 2-fold greater in patients with PD. Another retrospective study, by Weissman et al, showed that patients in whom PD was diagnosed by means of a semi-structured interview were at higher risk of reporting that they had had a heart attack. In prospective studies, patients with high phobic anxiety, a characteristic of PD, had a greater risk of sudden cardiac death or fatal coronary artery disease (or both). The potential causes of this association between greater cardiovascular risk and PD remain to be determined.

Elevated serum cholesterol levels have consistently been identified as a major conventional risk factor for cardiovascular disease, and elevated levels have been reported in most (although not all) studies of patients with PD. Consequently, elevated levels of serum cholesterol may be one reason why PD is associated with cardiovascular disease and death. Elevated levels of cholesterol in patients with PD are hypothesized to result from the neurobiological changes occurring during PAs. This hypothesis is supported by correlations between serum cholesterol levels and frequency of PAs and by observation of higher cholesterol levels in PD patients with more frequent and more intense PAs. The exact biological mechanism underlying this association between PAs and elevated cholesterol remains to be elucidated.

Acute mental stress is associated with an increase in serum cholesterol levels. Mental stress tests such as mental arithmetic or the Stroop Colour–Word Conflict test produce sustained elevation of blood pressure, heart rate and epinephrine. These changes are consistent with increased activity of the sympathetic nervous system after acute mental stress. McCann et al have suggested the stress-induced lipolysis model as a mechanism for the increase in sympathetic activity and the associated increase in serum cholesterol levels in response to acute mental stress. According to this model, epinephrine stimulates the release of free fatty acids (FFAs) from adipose tissue. These FFAs become available to the liver for the synthesis and secretion of very low density lipoprotein (VLDL) particles, which undergo modification in the circulation to become low-density lipoprotein (LDL) particles. The manufacture of VLDL particles from FFA, and their subsequent conversion to LDL particles, requires periods from hours to days. In support of this model, correlations have been observed between FFA levels and the magnitude of epinephrine increases in response to psychological stress. We suggest that similar events occur after a PA. We therefore hypothesize that the higher levels of cholesterol, particularly LDL cholesterol, described in patients with PD result from the release of FFAs in response to PAs and their subsequent conversion to LDL cholesterol.

Panicogenic agents such as the cholecystokinin B (CCK-B) receptor agonists pentagastrin and CCK-4 reliably induce panic symptoms in patients with PD and, to a lesser degree, in healthy volunteers. These panic symptoms are similar to those spontaneously experienced by PD patients during PAs. Challenges with pentagastrin and CCK-4 are therefore useful models for the investigation of PD. We have already shown that pretreatment with propanolol, a β-adren-
ergic antagonist, attenuates CCK-4-induced panic symptoms. These results suggest that CCK-B agonists induce panic symptoms, in part, through increased adrenergic or sympathetic activity. In accordance with these studies, as well as studies showing that acute mental stress results in the release of FFAs (likely through adrenergic activation), it is logical to hypothesize that pentagastrin challenge will result in time-dependent release of FFAs, which could explain the mechanism of correlations observed between serum cholesterol levels and frequency and severity of panic attacks in patients with PD.

In general, women have a lower risk of cardiovascular disease than men until menopause, when their risk approaches that observed in men. Despite recent controversies, hormone replacement therapy (HRT) significantly reduces cholesterol levels and cardiovascular death in postmenopausal women. Studies suggest that men with PD are at greater risk for cardiovascular disease and death than women with this disorder. A possible explanation for this difference between the sexes is that estrogen protects premenopausal women against the PA-induced increase in FFAs and the subsequent increase in LDL cholesterol. This hypothesis is supported by the observation that pretreatment with estrogen blunted the rise in FFAs occurring in response to mental arithmetic stress. We therefore hypothesized that a 3-day pretreatment with ethinyl estradiol (EE) would attenuate the anticipated pentagastrin-induced, time-dependent release of FFA.

Methods

We used a double-blind, crossover, placebo-controlled design with randomization of the order of a 3-day pretreatment with placebo (lactose) or EE (50 µg/d) to assess the effect of a 30-µg, 5-second intravenous bolus injection of pentagastrin in terms of intensity of panic symptoms and the release of FFAs into the plasma. We chose a relatively low dose of EE, since our intent was to assess the effect of a typical clinical dose, for example, that used in birth control pills. Our choice of a lower than usual dose of pentagastrin was justified by our objective of assessing whether pretreatment with EE had any effect; we therefore wanted to avoid an overwhelming effect of pentagastrin at the CCK-B receptor level.

Male PD patients and healthy male volunteers (HVs) were recruited through advertisements. Because of men’s low levels of endogenous progesterone, use of a male population to study the cardiovascular effect of estrogen minimizes the confounding effects of progesterone inherent in using a female population. Of the 34 subjects recruited, 6 (all patients with PD) withdrew between the initial screening visit and the first injection of pentagastrin (at a subsequent visit), and 3 underwent the first but not the second injection. The reasons for these withdrawals have been reported elsewhere. Fifteen patients with PD and 10 HVs between the ages of 20 and 48 years completed the study. The mean age of the PD patients was 32.5 (standard deviation [SD] 7.8) years, and that of the HVs was 27.5 (SD 8.1) years.

At visit 1 (V1), a diagnostic interview based on the Structured Clinical Interview for the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) was conducted to determine the eligibility of all subjects. In addition, each subject underwent electrocardiography (ECG), blood screening and physical examination. Eligible HVs had no current or lifetime personal history of an Axis I psychiatric disorder and no family history of PD. Eligibility of PD patients, with or without agoraphobia, was defined according to the diagnostic criteria specified in DSM-IV. The primary diagnosis required was PD. Subjects in both groups who completed the study, 6 had generalized anxiety disorder, 3 had social phobia, 1 had a specific phobia (fear of heights), 2 had obsessive–compulsive disorder, and 1 had post-traumatic stress disorder (related to a motor vehicle crash during adulthood). At V1, subjects were randomly assigned to receive a 3-day pretreatment with placebo before the first pentagastrin challenge at visit 2 (V2) and, a week later, a 3-day pretreatment with EE before visit 3 (V3), or vice versa. Each of V2 and V3 took place in the morning after 12 hours of fasting. In addition, subjects were not allowed to smoke or have any caffeine on the morning of the...
pentagastrin challenge. They were also told to refrain from alcohol for the 24-hour period before the pentagastrin challenge. On the morning of each of the 3 pretreatment days (the third day being the injection visit), a pretreatment tablet was taken at a time 1 hour earlier than the designated time of arrival for the injection visit. These times were kept consistent for V2 and V3. None of the subjects reported side effects induced by either of the pretreatments.

Upon arrival at V2 and V3, each subject was seated in a reclining chair, and an intravenous catheter, through which a 0.9% NaCl solution was run at 125 mL/h, was installed in the right antecubital vein. The subject remained in this semisupine position for the duration of the procedure. The time of intravenous installation was recorded as t = –45 minutes (i.e., 45 min before administration of pentagastrin). At t = 0 minutes, the 30-µg dose of pentagastrin was administered as a 5-second intravenous bolus injection through a 3-way valve connected to the indwelling intravenous catheter. At t = 5 minutes, the subject’s panic symptoms were evaluated according to an 18-item Panic Symptom Scale (PSS) derived from the DSM-III-R. Subjects were asked to rate the severity of their symptoms on a scale from 0 to 4 (0 = absent, 1 = mild, 2 = moderate, 3 = severe, 4 = extremely severe). Scores were summed to yield a PSS score. At t = –10 minutes and at t = 1.5, 6, 30, 60 and 120 minutes, blood samples were obtained for measurement of FFA. Of a possible total of 150 samples, 23 were missing because of technical difficulties. All statistical analyses were based on the samples available, on the assumption that the missing samples were missing at random.

FFA analyses were performed in an independent analytical laboratory according to the following procedure. The serum sample was added to acyl-CoA synthetase in the presence of adenosine triphosphate, magnesium ions and CoA at 37°C, to form thiol esters of CoA as well as the byproducts adenosine monophosphate and pyrophosphate. Because ascorbic acid is known to interfere with the hydrogen peroxide produced during the reaction, ascorbic acid in the serum sample was removed by adding ascorbate oxidase to the reaction mixture. The acyl-CoA esters produced were further oxidized by the addition of acyl-CoA oxidase to produce hydrogen peroxide, which, in the presence of peroxidase, allows oxidative condensation of 3-methyl-N-ethyl-N(β-hydroxy-ethyl)-aniline with 4-aminoantipyrine to form a purple adduct that is read colorimetrically. The amount of free acids in the sample is directly proportional to the intensity of the colour produced.

Informed consent was obtained from each subject, and monetary compensation was provided for participation in the study. The study was approved by the Health Research Ethics Board of the University of Alberta in Edmonton.

Statistical analysis

A linear model for crossover design was used to analyze the data. The following parameters were included in the model: the main effects of time, diagnosis (HV or PD patients), pretreatment (EE or placebo), visit (V2 or V3) and order of pretreatment (EE then placebo or placebo then EE) and the interactions of diagnosis with time, diagnosis with pretreatment, diagnosis with visit, diagnosis with order of pretreatment, diagnosis with pretreatment and time, diagnosis with visit and time, diagnosis with order of pretreatment and time, pretreatment with time, visit with time, and order of pretreatment with time. For our study, p values less than 0.05 were considered significant. Bonferroni’s multiple-comparisons analyses were also performed where appropriate.

Results

PD patients presented with a greater severity of panic symptoms than HVs (mean PSS scores 35.0 [SD 14.7] v. 22.4 [SD 8.9], F_{1,21} = 9.58, p = 0.006). Neither pretreatment (F_{1,21} = 0.20, p = 0.66), diagnosis with pretreatment (F_{1,21} = 0.18, p = 0.67), order of pretreatment (F_{1,21} = 1.24, p = 0.28) nor visit (F_{1,21} = 1.33 p = 0.26) had any effect on the pentagastrin-induced PSS score.

After challenge with pentagastrin, there was a statistically significant effect of time on serum FFA levels (Fig. 1), which was not affected by pretreatment, order of pretreatment or visit; in fact, no interactions with time were statistically significant (Table 1). Similarly, we found no diagnosis main effect and no interaction between diagnosis and time, although the graphic results suggested a lower release of FFAs in PD patients. We found no significant interactions involving diagnosis, pretreatment or order of pretreatment. Post hoc analysis with the Bonferroni adjustment for multiple comparisons revealed a statistically significant increase in average FFA levels, relative to baseline, at 2
hours after pentagastrin administration ($t_{100} = 4.52$, adjusted $p < 0.001$).

**Discussion**

The main finding of the present study was that administration of pentagastrin results in a time-dependent release of FFAs in both HVs and patients with PD. This finding is consistent with other research in which FFAs have increased in response to mental stress. However, mental stress and PAs are not equivalent, and, to our knowledge, this is the first human study to examine the release of FFAs in response to a panicogenic challenge. Our previous research suggests that increased adrenergic activity, from CCK-B agonists, plays a role in inducing panic symptoms. It is possible, therefore, that CCK-B agonists cause a time-dependent release of FFAs from adipocytes through sympathetic activation. This would support our hypothesis that the higher levels of LDL cholesterol in PD patients result from activation of the sympathetic nervous system during PAs, release of FFAs from adipocytes and subsequent conversion of the FFAs to LDL cholesterol.

PD patients demonstrated greater sensitivity to challenge with pentagastrin than HVs, an observation consistent with other studies where this finding has been discussed extensively. There was no effect of pretreatment with EE on PSS scores, which confirms preliminary findings published and discussed elsewhere. The lack of pretreatment effect with EE is in accordance with the lack of consistency in reports of the effect of estrogen-based compounds on panic symptoms in PD patients.

The model we have discussed suggests that PAs and the resultant increases in FFAs account for elevated serum cholesterol levels in PD patients. Given this model, as well as the effects of estrogen in lowering cholesterol in postmenopausal women, we hypothesized that pretreatment with EE would attenuate the rise in FFAs in response to pentagastrin challenge. In fact, pentagastrin-induced release of FFAs was not affected by pretreatment with EE. This result is contrary to a previous study, which showed that pretreatment with estrogen prevented the increase in FFAs that otherwise occurs in response to mental arithmetic stress. One possibility for this discrepancy could be a difference in dose or route of administration of estrogen (100 µg administered percutaneously in the previous study and 50 µg administered orally in our study). Nonetheless, we know that the dose we used can have physiological effects, because this dose reduced a pentagastrin-induced increase in heart rate in a previous study. Furthermore, given that the dose we used is typical of HRT doses and given that HRT has cardio-protective effects in postmenopausal women, it is possible that estrogen works through a different mechanism to exert its cholesterol-lowering effects.

The finding of a pentagastrin-induced increase in FFAs in this study has a number of important implications. First, it supports our hypothesis that what occurs during PAs follows a model similar to the stress-induced lipolysis model described by McCann et al. This mechanism would explain the elevated serum cholesterol levels in PD patients.

<table>
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Note: df = degrees of freedom.
cholesterol levels in PD patients. It might also explain some of the discrepancies (i.e., a mix of positive and negative studies) among investigations of cholesterol levels in patients with PD. According to our proposed mechanism of action for elevation of cholesterol levels in patients with PD, patients experiencing daily PAs would display higher levels of FFAs daily and subsequent daily increases in serum LDL cholesterol concentrations. In contrast, cholesterol levels in patients with infrequent PAs would likely remain unchanged, because cholesterol measurement would be less likely to take place soon after a PA and therefore would not be preceded by an increase in serum FFA levels. Thus, patients with daily PAs would probably have chronic elevation of cholesterol levels, and patients with infrequent PAs would not.

The increase in serum cholesterol is itself a major conventional risk factor for cardiac disease. However, the rise in FFAs induced by PAs may account for the greater cardiovascular mortality rate in PD patients through other mechanisms as well. An infusion of FFAs in HVs resulted in decreased heart-rate variability, and it has recently been shown that decreased heart-rate variability correlates with an increased risk of sudden death. Indeed, decreased heart-rate variability has been demonstrated in patients with PD. Therefore, this demonstration that induced panic attacks result in the release of FFAs suggests 2 possible underlying mechanisms for the greater cardiovascular mortality rate in PD patients.

We acknowledge some limitations inherent in the current study. Some of the PD patients had comorbid disorders and therefore may not perfectly reflect patients with PD only. Because our study lacked a placebo injection visit, it is possible that some of the changes in FFAs were due to stress experienced as a result of the injection, rather than to the pentagastrin. As well, samples of blood for the measurement of FFAs were collected over a 2-hour period. This time frame was not optimal, in that we stopped collecting samples before FFA level peaked (see Fig. 1). Although it would be logical and very likely, we have not shown that such a peak is followed by an increase in LDL cholesterol levels. To confirm our current results, we will need to demonstrate an increase in serum LDL cholesterol 24 hours after the pentagastrin-induced increase in FFAs. In future studies, we plan to increase the dose of pentagastrin, include a placebo injection visit, measure serum cholesterol after 24 hours and expand the time course of blood collection.

In conclusion, the present study indicates that pentagastrin-induced PAs result in a time-dependent release of FFAs. This result may explain the elevated serum cholesterol levels observed in patients with PD. It will be important to show that this panic-induced increase in serum FFA levels is indeed followed by an increase in LDL cholesterol concentrations. Further studies are needed to assess the consequences in terms of cardiovascular risk.

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References


