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THE INFLUENCE OF FEMALE HORMONES AND OTHER NEUROACTIVE STEROIDS ON PANIC DISORDER

by

GLENDON RALPH TAIT 

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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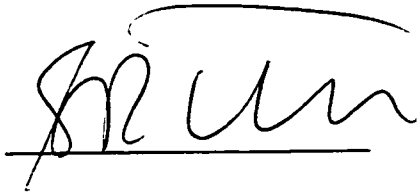

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled: THE INFLUENCE OF FEMALE HORMONES AND OTHER NEUROACTIVE STEROIDS ON PANIC DISORDER submitted by Glendon Ralph Tait in partial fulfilment of the requirements for the degree of Master of Science.

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DEDICATION

This thesis is dedicated to the memory of Dr. Peter McGahan who taught me the importance of training the mind not just in preparation for a profession, but as an end unto itself.

ABSTRACT

Challenge with the cholecystokin type B (CCK_B) agonist pentagastrin is a useful tool for investigating the pathophysiology of panic disorder (PD). This thesis examined the effect of estrogen on the panic response and cardiovascular reactivity following pentagastrin challenge and the effect of progesterone on the panic response. In addition, levels of female hormone-derived neuroactive steroids (NASs) were compared between healthy volunteers (HVs) and panic disorder (PD) patients at baseline and following challenge. Though exogenous estrogen had no effect on the panic response, it reduced cardiovascular reactivity. Progesterone attenuated the pentagastrin-induced panic response, its anxiolytic activity likely mediated through its metabolite allopregnanolone (ALLO). Though PD vs HV did not differ at baseline, after panic challenge PD patients appeared to have a decreased ability to release ALLO; in addition there was a release of dehydroepiandrosterone (DHEA). This is the first human study to observe changes in progesterone-derived NASs following a stressful challenge.

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ABBREVIATIONS

ACN	acetonitrile
ALLO	allopregnanolone
BDZ	benzodiazepine
BP	blood pressure
bpm	beats per minute
CCK	cholecystokinin
Cl^-	chloride
CO_2	carbon dioxide
DBP	diastolic blood pressure
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
ECG	electrocardiogram
EE	ethinyl estradiol
GABA	gamma-aminobutyric acid
GAD	generalized anxiety disorder
h	hour (s)
5-HT	5-hydroxytryptamine
Hg	mercury
HR	heart rate
HRT	hormone replacement therapy
HV	healthy volunteer
i.v.	intravenous
MAOI	monoamine oxidase inhibitor
mg	milligram (s)
min	minute (s)
mm	millimeter (s)
MDE	major depressive episode
MP	medroxyprogesterone
NA	noradrenaline
NaCl	sodium chloride
NAS	neuroactive steroid
ng	nanogram (s)
OCD	obsessive compulsive disorder
p	probability
PD	panic disorder
PA	panic attack
Pl	placebo
PREGS	pregnenolone sulfate
PSS	panic symptom scale
PTSD	post-traumatic stress disorder
$[^{35}S]$TBPS	t-$[^{35}S]$-butylbicyclophosphorothionate
SBP	systolic blood pressure

s	second (s)
SSRI	selective serotonin reuptake inhibitor
t	time
TCA	tricyclic antidepressant
μg	microgram
V	visit

CHAPTER 1

General Introduction: The Influence of Female Hormones and other Neuroactive Steroids on Panic Disorder

1.1. Introduction

Panic disorder (PD) is an anxiety disorder that affects 1% - 3% of the population (Weismann et al., 1997). The defining characteristic of PD is the repeated occurrence of panic attacks (PAs). These attacks are composed of psychological symptoms (including anxiety, fear of dying, fear of losing control, etc.) and physical symptoms (shortness of breath, heart palpitations, chest pain or pressure, etc.) (DSM-IV, American Psychiatric Association, 1994). Agoraphobia is a frequent complication of PD, affecting two-thirds of PD patients. Agoraphobia consists of avoidance of places or situations in which escape or help are potentially unavoidable in the event of a PA (Brier et al., 1986; Dick et al., 1994; Magee et al., 1996). Because the symptoms of PD mimic those of many medical conditions, PD patients often assess their PAs as being associated with potentially life-threatening events, leading to visits to the emergency room and help-seeking behavior. Medical evaluations, sometimes numerous, result in high health care utilization costs (Salvador-Carulla and Fernandez-Cano, 1995; Shelbourne et al., 1996). Treatment for PD includes pharmacological treatment with tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), benzodiazepines (BDZs), and monoamine oxidase inhibitors (MAOIs), as well as cognitive behavior therapy (Katon, 1997). Within one year of treatment, less than one-third of PD patients reach full remission (Keller et al., 1994). Thus PD is clearly a disabling disorder which is not optimally treated.

CCK, the most abundant neuropeptide in the human brain, has receptors distributed widely throughout the central nervous system (Zarbin et al., 1983; Vandijk et

al., 1984; Hill et al., 1987). There are two receptor subtypes, CCK_A and CCK_B (Moran et al., 1986; Dourish et al., 1987; Wank et al., 1992), recently renamed CCK_1 and CCK_2 (Noble et al., 1999). In the brain, the latter is the most common subtype (Vandijk et al., 1984). CCK is suspected to play a major role in PD. For example, genetic abnormalities of the coding of the CCK_B receptor have been identified in PD patients (Miyamoto et al., 1996; Kennedy et al., 1997). In addition, CCK has been shown to interact with other neurotransmitter systems suspected in the pathophysiology of PD, including gamma-aminobutyric acid (GABA), noradrenaline (NA), and serotonin (5-hydroxytryptamine, 5-HT) (VanMegen et al., 1996). Another major argument in favor of the involvement of CCK in PD is the fact that CCK_B agonists such as CCK-4 and pentagastrin induce panic symptoms in PD patients and, to a lesser extent, in HVs (Bradwejn et al., 1991a,b; Abelson and Nesse, 1994). CCK_B agonists fulfill all characteristics of a panicogenic agent, being safe for human use; inducing emotional and somatic symptoms of a PA; reproducing symptoms of spontaneous attacks; displaying specificity for this disorder; showing dose-dependent and reproducible effects; and being antagonized by antipanic agents while not being antagonized by non-antipanic agents (Bradwejn et al., 1993).

Female hormones have been suspected to play a role in the pathophysiology of PD since this disorder occurs twice as frequently in females as in males, while PD with agoraphobia is three times as frequent in women as in men (DSM-IV). Natural fluctuations in hormones seem to affect the symptomatology of PD. While some retrospective and prospective studies on women with PD found worsening of panic symptoms during the premenstrum, a period associated with decreases in progesterone,

(Brier et al., 1986; Sandberg et al., 1986; Kaspi et al., 1994), others did not (Cameron et al., 1988; Stein et al., 1989). Although pregnancy, with its increase in estrogen and progesterone, seems to improve the course of PD (George et al., 1987; Cowley et al., 1989 Kraus et al., 1989; Cohen et al., 1994), this was not observed in one small pilot prospective study (Cohen et al., 1996) which included severely ill and pharmacologically treated PD patients. In another study, our team had one subject whose mechanical contraception method failed; she became pregnant between two pentagastrin injections and demonstrated a much smaller response during the injection visit which took place during pregnancy (LeMellédo et al., unpublished results). The post-partum, associated with an abrupt decrease in estrogen and progesterone, seems to elicit or worsen PD (Metz et al., 1988; Klein et al., 1993 Cohen et al., 1994;). Findings of PD improving when progesterone is high or worsening when it is low may be explained by the anxiolytic effect of progesterone, well established in animal models of anxiety (Bitran et al., 1995).

In some anecdotal reports, estrogen-replacement therapy has been shown both to improve (Korhonen et al., 1995) and worsen (Price et al., 1988; Dembert et al., 1994) PD symptomatology. Evidence suggests that PD patients exhibit an increased risk for cardiovascular death (Coryell et al., 1982,1986; Kawachi et al., 1994). Despite PD occurring more frequently in females, male PD patients have been shown to be at an increased risk from cardiovascular death, compared to females (Coryell et al., 1982, 1986). The cardioprotective effects of estrogen, possibly through altering the cardiovascular response to a PA, may explain the gender difference in cardiovascular mortality in PD patients. For ethical and practical reasons, prospective investigation of

the effects of exogenously administered estrogen on PD symptomatology would not be feasible. However, challenge with the CCK_B agonist pentagastrin is a useful tool for investigating the pathophysiology of PD in a controlled laboratory setting. Given the likely role for female hormones in PD, this thesis investigated, separately, the effect of pretreatment of exogenously administered estrogen and progesterone, compared to placebo, on pentagastrin-induced behavioral panic symptoms as well as the effect of estrogen pretreatment on pentagastrin-induced cardiovascular reactivity. Though it is not clear how estrogen might affect PD symptomatology, progesterone displays anxiolytic activity in animal models of anxiety (Bitran et al., 1995), likely through its metabolite ALLO, a NAS.

NASs encompass all steroidal compounds that exert effects in neural tissue, whether they are synthesized in the nervous system or peripherally in the endocrine glands (Orchinik and McEwen, 1993). NASs have been shown to exert effects at receptor complexes implicated in the pathophysiology of human anxiety, including the inhibitory $GABA_A$ receptors and the excitatory glutamate receptors. Neuromodulation of the $GABA_A$/BDZ receptor can be accomplished by NASs (Majewska et al., 1986; Majewska et al., 1992; Paul and Purdy, 1992). ALLO, a positive neuromodulator of the $GABA_A$/BDZ receptor, appears to be responsible for the anxiolytic activity of progesterone observed in animal studies since inhibition of the enzyme responsible for the conversion of progesterone to ALLO blocks the anxiolytic activity of progesterone (Bitran et al., 1993; Bitran et al., 1995; Reddy and Kulkarni, 1996). Since the $GABA_A$/BDZ receptor modulates output to many systems involved in anxiety, it is

reasonable to suspect that alterations at this site may affect human anxiety disorders (Mailizia et al., 1995). Barbaccia et al. (1996a,b) found a delayed release of NASs in rats following CO_2 inhalation, a procedure which induces acute stress in animals and PAs in humans. The delayed release of NASs, particularly positive allosteric modulators such as ALLO, may be an endogenous homeostatic mechanism which seeks to restore tone to a GABAergic system disrupted by stress. There have, however, been no human studies to investigate the role of NASs in PD. One focus of this thesis research was thus to determine whether PD patients, relative to HVs, differ at baseline, on NAS levels and whether pentagastrin challenge would induce changes in levels of NASs.

In sum, this thesis investigated the following:

- the effect of estrogen, compared to placebo, on pentagastrin-induced behavioral panic symptoms and pentagastrin-induced cardiovascular reactivity; it was hypothesized that pretreatment with estrogen would alter the behavioral and cardiovascular components of the panic response to pentagastrin.
- the effect of progesterone, compared to placebo, on pentagastrin-induced panic symptoms; it was hypothesized that pretreatment with progesterone would decrease the panic response to pentagastrin
- baseline differences in NASs between HVs and PD; it was hypothesized that baseline plasma levels of NASs would be altered in medication-free PD patients when compared to HVs.
- pentagastrin-induced release of NASs in PD and HVs pretreated with placebo or

ethinyl estradiol; it was hypothesized that PD patients would show an augmented release of NASs because of their expected greater panic response. It was further hypothesized that pretreatment with ethinyl estradiol would affect the panic response to pentagastrin and would alter the associated release of NASs

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CHAPTER 2

Effect of Ethinyl Estradiol on the Panic Response to the Panicogenic Agent Pentagastrin*

*The contents of this chapter are represented in a manuscript which is in press in the Journal of Affective Disorders (K McManus, **GR Tait**, F Bellavance, J-M Le Mellédo). The author of this thesis played a major role in this study including design and revision of protocol, research visits, data collection, and revision of the manuscript.

2.1 . Introduction

PD with or without agoraphobia is an anxiety disorder which afflicts 1% - 3% of the general population (Weissman et al., 1997). The primary symptom of PD is the repeated occurrence of PAs, characterized by psychological symptoms (anxiety, fear of dying, losing control or going crazy) and physical symptoms (shortness of breath, heart palpitations, chest pain or pressure, trembling, nausea) (DSM-IV, American Psychiatric Association, 1994). PD occurs 2-3 times more frequently in women than in men. In female PD patients, hormonal events associated with fluctuations in levels of female hormones, such as estrogen, have been reported to alter the course of PD. These events include the premenstrual period of the menstrual cycle, pregnancy, menopause, oral contraceptives and hormone replacement therapy (HRT) (Brier et al., 1986; Price and Heil, 1988; Cowley and Roy-Byrne, 1989; Deci et al., 1992; Ushiroyama et al., 1992; Kaspi et al., 1994; Northcott and Stein, 1994; Korhonen et al., 1995). The data reported from these studies are, however, somewhat inconsistent, with some observing an improvement and others reporting an onset or worsening of PD symptomology following the initiation of oral contraceptives (Deci et al., 1992; Ushiroyama et al., 1992) and HRT (Price and Heil, 1988; Korhonen et al., 1995).

Despite methodological limitations of epidemiologic studies on cardiovascular risk associated with PD, research findings have suggested that PD patients exhibit an increased risk for cardiovascular death (Coryell et al., 1982,1986; Kawachi et al., 1994). The increased cardiovascular risk described in PD patients might be associated with PA-

related arrhythmia and hyperventilation which can induce coronary vasoconstriction (Esler, 1998; Mansour et al., 1998). Indeed, in this patient population arrhythmia and coronary vasoconstriction have been suggested to be the main causes of cardiac death, particularly sudden cardiac death (Kawachi et al., 1994, 1995; Kuzbansky et al., 1998).

Although PD occurs more frequently in women, studies suggest that PD males are at an increased risk for cardiovascular disease and/or cardiovascular death compared to PD women (Coryell et al., 1982, 1986). In women, menopause is associated with an increased risk for cardiovascular disease which approaches that observed in men (Barrett-Connor, 1997). Numerous observational studies have shown the cardioprotective effect of exogenous estrogens when administered alone (for review see Grodstein et al., 1995). For example, it has been shown that HRT in postmenopausal women significantly reduces cholesterol levels and cardiovascular mortality (Stampfer et al., 1985; Henderson et al., 1991; Stampfer and Colditz, 1991; Grodstein et al., 1996). Recent findings in the HERS study (Heart Estrogen/Progestin Replacement Study) have, however, failed to show any significant cardioprotective effect of combining estrogens and progestins in HRT compared with placebo in postmenopausal women (Hulley et al., 1998). The interference of progestins with the cardioprotective effects of estrogens may explain the negative findings of this study (Sarrel, 1995; The Writing Group for the PEPI Trial, 1995; Miyagawa et al., 1997; Mercuro et al., 1999). The HERS study is also limited by the fact that women enrolled in this study had established coronary disease.

It has been estimated (Barrett-Connor, 1990) that only 30% of the reduction in the risk of cardiovascular disease can be explained by the cholesterol-lowering properties of

estrogen replacement therapy. There is evidence (Lindheim et al., 1992; Del Rio et al., 1994, 1998; Sita and Miller, 1996) that administration of female sex hormones such as ethinyl estradiol (EE) can reduce the cardiovascular response to acute stress. It is, therefore, reasonable to hypothesize that estrogens may decrease cardiovascular reactivity during PAs.

Due to the inherent difficulties of studying the influence of female hormones on panic symptomology during the natural course of PD, this study assessed the effects of estrogen on a pentagastrin-induced panic response in a controlled laboratory setting. Pentagastrin, a CCK_B receptor agonist, is a panicogenic agent which reliably induces PAs in PD patients (and to a lesser degree in HVs) similar to those spontaneously experienced by PD patients (Abelson and Nesse, 1994; van Megen et al., 1994). Consequently, panic challenges with agents such as pentagastrin serve as useful tools with which to study the pathophysiology of PAs. The present investigation sought to study the effects of EE on pentagastrin challenge in male PD patients and HVs. Because of their low levels of progesterone, using a male population to study the cardiovascular effect of estrogen avoids the confounding effects inherent in using progesterone in a female population (Mercuro et al., 1999). It was hypothesized that a 3-day pretreatment with EE would alter the behavioural and cardiovascular components of the panic response to pentagastrin. Therefore, the pentagastrin-induced panic responses following placebo (Pl) and EE pretreatment were compared. For this comparison, the panic sum intensity was measured using the panic symptom scale (PSS) as well as pentagastrin-induced increases in heart rate (ΔHR) and systolic (ΔSBP) and diastolic (ΔDBP) blood pressure (BP).

2.2. Materials and Methods

2.2.1 Design and Drugs Used

A double-blind cross-over placebo-controlled design with randomization of the order of a 3-day pretreatment of placebo (lactose) (Odan Laboratories, Montréal, P.Q. Canada) or EE (Estinyl ®) (Schering, Montréal, P.Q., Canada)(50mg/day) was used to assess the effect of a 30-mg 5-sec i.v. bolus injection of pentagastrin (Peptavlon®, Wyeth-Ayerst, Philadelphia, PA or Clinalfa, Läufelfingen, Switzerland) on the panic symptom intensity and the increase in HR, DBP and SBP following each pretreatment. The production of pentagastrin by Wyeth-Ayerst was discontinued after the study had begun so it was necessary to continue the study using pentagastrin from Clinalfa. There was no difference, however, between the effects observed with pentagastrin obtained from one provider versus the other.

2.2.2. Subjects

Of the subjects recruited, three (all PD patients) withdrew between the initial screening visit and the first pentagastrin injection visit. One decided not to continue the study upon arrival for the first injection visit, and the other two could not be reached to be rescheduled. Two other PD patients received the first, but not the second, pentagastrin injection. One patient (whose pretreatment for the first injection was EE) could not be reached to arrange his second injection visit. The other patient (whose pretreatment for the first injection was placebo), fainted during the first injection visit so he was

withdrawn from the study. Indeed, rare occurences of vasovagal reactions have been described by other authors (Bradwejn et al., 1992). Subjects who completed the study were 11 male PD patients and 9 male HVs between the ages of 21 and 48 years old. The mean ages of the PD patients and the healthy controls were 33.7 ± 8.1 and 27.4 ± 8.5 years, respectively.

At visit 1 (V1), a diagnostic interview, using the Structured Clinical Interview for DSM-IV (SCID) (American Psychiatric Association 1994), was conducted to determine the eligibility of both PD patients and healthy controls. Additionally, an electrocardiogram (ECG), blood screening and physical examination of each subject were carried out. To meet the criteria for a HV, subjects must have had no current or lifetime personal history of an Axis-I psychiatric disorder. They were also required to have no family history of PD. Subjects (PD patients or HVs) were excluded from the study if they were heavy smokers (>15 cigarettes/day), heavy coffee drinkers (>5 cups/day) or recreational drug users. All subjects were required to be medication/drug-free during the study, and PD patients were not to have taken psychotropic medication in the 2 months prior to V1. In addition, they were excluded from participating in the study if medical assessment, blood screening or ECG indicated that they were ill or at risk due to the pentagastrin injection. Eligibility of PD patients, with or without agoraphobia, was defined according to the criteria specified in DSM-IV (American Psychiatric Association, 1994). The primary diagnosis was required to be PD; however, because of the within-subjects design and the frequent comorbidity in PD (Katerndahl and Realini et al., 1997; Kaplan and Sadock, 1998), subjects were included in the study even if they had a

secondary anxiety disorder such as generalized anxiety disorder (GAD), social phobia, specific phobia, obsessive-compulsive disorder (OCD) or post-traumatic stress disorder (PTSD). Of the 11 PD patients who completed the study, six had GAD, two had social phobia, one had specific phobia (fear of heights), two had OCD, and one had PTSD (related to a motor vehicle accident during adulthood).

2.2.3. Research Visits

At V1, subjects were randomly assigned to receive a 3-day pretreatment of either placebo prior to the first pentagastrin challenge at visit 2 (V2) and, a week later, EE prior to visit 3 (V3), or vice versa. Each of these two visits (V2 and V3) took place in the morning following 12 h of fasting. In addition, subjects were not allowed to smoke or have any caffeine the morning of the pentagastrin challenge. They were also told to refrain from alcohol for the 24-h period prior to the pentagastrin challenge. On the morning of each of the 3 pretreatment days (the 3rd day being the injection visit), a pretreatment tablet was taken at a time chosen to be 1 h earlier than the designated time of arrival for the injection visit. These times were kept consistent for V2 and V3. None of the subjects complained of side effects induced by EE or placebo.

Upon arrival at V2 and V3, subjects were seated in a reclining chair and an i.v. catheter, through which a 0.9% NaCl solution was run at 125 ml/h, was installed in their right antecubital vein. They remained in the semi-supine position for the duration of the procedure. Arrival time and, consequently, i.v. installation time (t) was recorded as t = -45 min (45 min prior to the time of pentagastrin administration). Three diaphoretic

monitoring electrodes (ECG leads) were placed on the subjects' chest and HR was measured with continuous ECG recording. DBP and SBP were monitored with an automatic sphingomanometer (BP cuff placed on left arm) and recorded automatically using a Dynamap® monitor (Critikon, Canada) starting at t = -45 min until t = -20 s, after which these vital signs were recorded automatically every 20 s until 5 min after the injection. At t = -5 min, subjects were told not to speak from that point until 5 min after the pentagastrin injection since speaking may alter HR (Liehr, 1994). At t = 0, pentagastrin was administered as a 30-mg 5-s i.v. bolus injection through a three-way valve connected to the indwelling i.v. catheter. For HR, SBP and DBP measures, we computed the maximum increase from baseline, i.e. maximum value following injection minus baseline value (value obtained just before injection).

At t = +5 min, the subjects' panic symptoms were evaluated using an 18-item DSM-III-R-derived Panic Symptom Scale (PSS) (Bradwejn et al., 1991). 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. 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, Edmonton, Alberta, Canada.

2.2.4. Statistics

The main objective was to determine whether EE, compared to placebo, had any effect on pentagastrin-induced behavioural (PSS scores) and cardiovascular (ΔHR, ΔSBP and ΔDBP) responses in PD patients and HVs. Following the experimental design, a linear model for cross-over design (Jones and Kenward, 1989) was used to analyse the data. The following parameters were included in the model: the main effect of diagnosis (HVs vs. PD patients), the sequence in which the subjects received the pretreatment (EE prior to the first pentagastrin injection and Pl prior to the second pentagastrin injection, or vice versa, i.e. EE/Pl or Pl/EE), the main effect of pretreatment (EE vs. Pl), the main effect of visit (V2 or V3), and the interactions of diagnosis with sequence, diagnosis with pretreatment and diagnosis with visit. *P* values less than 5% were considered significant. All statistical analyses were conducted using SAS statistical software for Windows, version 6.12.

2.3. Results

The mean scores ± standard deviation (SD) for PSS, ΔHR, ΔSBP and ΔDBP obtained after pentagastrin injection for each diagnosis and pretreatment are described in Table 1. PD patients, as compared to HVs, had greater panic symptom severity as indicated by PSS scores [F(1,16)=8.45; p=0.01]. Pretreatment with EE significantly reduced the pentagastrin-induced ΔHR in both PD patients and HVs (see figure 1). PSS score [F (1,16) = 0.04, p=0.84], ΔSBP [F(1,16)=1.35; p=0.26] and ΔDBP [F(1,16)=1.11; p=0.31] were not significantly altered by pretreatment. The order in which pretreatment

(EE/Pl or Pl/EE) was given had no significant effect on the behavioural or cardiovascular responses to pentagastrin, as indicated by the absence of a sequence effect for PSS score [F (1,16)=0.14, p= 0.71], ΔHR [F(1,16)= 0.61: p=0.45], ΔSBP [F(1,16)=1.61, p=0.22] and ΔDBP [F(1,16)=0.27, p=0.61].

2.4. Discussion

The main findings of the present study are that EE pretreatment attenuated the pentagastrin-induced increase in HR and did not alter the behavioural response to pentagastrin or the pentagastrin-induced increase in SBP and DBP.

Compared to HVs, the increased sensitivity of PD patients to pentagastrin reported here is consistent with other studies (Abelson and Nesse, 1994; van Megen et al., 1994) which have also reported a greater behavioural response by PD patients versus controls to pentagastrin. This is thought to be the first study to assess the effects of administration of female hormones on a panic response. As a consequence, there are no panic studies for comparison. Although panic challenges are not equivalent to mental stress tests, it is interesting to compare the present results to investigations of the effects of estrogens on the response to mental stress tests [e.g.Stroop Colour-Word Conflict (Stroop), mental arithmetic, public speaking, etc.]. The dissociation observed in the present study between the behavioural and cardiovascular response is consistent with results obtained by Del Rio et al. (1994). These authors observed that, in healthy men, pretreatment with EE (100mg transdermal patch applied 24 h prior to testing) failed to attenuate the behavioural response to mental arithmetic stress as measured by state

anxiety (Spielberger) scores. They did, however, find a reduction by EE of the stress-induced increase in HR.

The possibility cannot be excluded that the lack of effect of EE on the behavioural response is due to the low dose of EE that was administered. A relatively low dose of EE was chosen since the purpose was to assess the effect of an average dose of EE used clinically, for example that which is used in birth control pills. Pharmacokinetic studies have shown that plasma EE levels are in the 200 μg/ml range after oral administration of 50 mg of EE. By comparison, 200 μg/ml is the average concentration of endogenous plasma estradiol levels during the luteal phase in women.

Estrogens have rapid non-genomic effects as well as less rapid genomic effects. It is likely that a 3-day pretreatment is sufficient to also obtain genomic effects (Mendelsohn and Karas, 1999). The pretreatment period was limited to 3 days due to ethical concerns (potential thromboembolic risk) and feasibility (male subjects may have been reluctant to take female hormones long-term).

Alteration of the beta-adrenergic system by EE may explain the current results. Indeed, estrogen has been shown to reduce beta-adrenergic responsiveness in rats (Black et al., 1976). It has been previously shown by our research team that HR response to a CCK_B agonist (CCK-4) is at least partially mediated through the beta-adrenergic system (Le Mellédo et al., 1998). Indeed, our team showed that pretreatment with propranolol, a beta-adrenoceptor antagonist, decreases the HR reactivity to CCK-4 and that there is a negative correlation between the HR reactivity to CCK-4 and the dose of isoproterenol necessary to increase HR by 25 beats per minute (bpm) (reflecting beta-adrenergic

receptor senstivity) (Le Mellédo et al., 1998). In further support of an adrenergic-mediated effect of EE on pentagastrin-induced increase in HR, Del Rio et al. (1994, 1998) have demonstrated that the increased plasma adrenaline levels elicited by mental stress (arithmetic or Stroop test) in both men and postmenopausal women is reduced by EE pretreatment.

Due to its reported effects on cardiovascular and neuroendocrine responses, psychological or mental stress has been found to contribute to an individual's risk for cardiovascular disease (Krantz and Manuck, 1984). Attenuation of acute cardiovascular reactivity to various stressors following pretreatment with estrogen, combined with evidence of increased cardiovascular death in male PD patients, is consistent with the present findings of a decreased cardiovascular reactivity during a panic challenge in male PD patients pretreated with EE. Together these data suggest a role for EE as having acute cardioprotective effects during PAs.

One of the main causes of sudden cardiac death is arrhythmia, a phenomenon directly under the influence of adrenergic activity (Kawachi et al., 1994, 1995). Therefore, one potential mechanism of estrogen's protective effect could be a decrease in arrhythmic events occurring during PAs, with this effect being mediated through an estrogen-induced decrease in adrenergic activity (resulting in a consequent increase of the arrhythmia threshold).

In conclusion, the present study has demonstrated that EE pretreatment significantly attenuated the HR reactivity induced by the panicogenic agent pentagastrin in males. This observation may be of considerable importance in light of the fact that

studies have suggested that the increased risk of cardiovascular death in PD patients is greater in males. Estrogen may serve an important cardioprotective role in the context of an acute panic attack in PD patients, but studies are needed to assess the effect of estrogens on the panic response to pentagastrin in female PD patients.

	Healthy Volunteers (n = 9)		Panic Disorder Patients (n = 11)	
	PI	EE	PI	EE
PSS score	24.11 ± 9.03	21.89 ± 9.74	37.73 ± 15.69	39.27 ± 15.27
Δ HR	40.44 ±12.26	35.89 ± 16.10*	36.91 ± 14.05	30.45 ± 12.26*
ΔSBP (mm Hg)	18.78 ± 18.23	23.00 ± 9.50	21.36 ± 6.58	20.55 ± 5.79
ΔDBP (mm Hg)	11.89 ± 4.46	10.56 ± 6.75	12.09 ± 4.83	10.73 ± 5.44

Table 1: Means and standard deviations of the Sum Intensity of Panic Symptoms (PSS score) and maximum increases in Heart Rate (ΔHR), Systolic Blood Pressure (ΔSBP), and Diastolic Blood Pressure (ΔDBP) after administration of pentagastrin to HVs and PD Patients according to pretreatment. Significance ($p<0.05$) is represented by an asterisk.

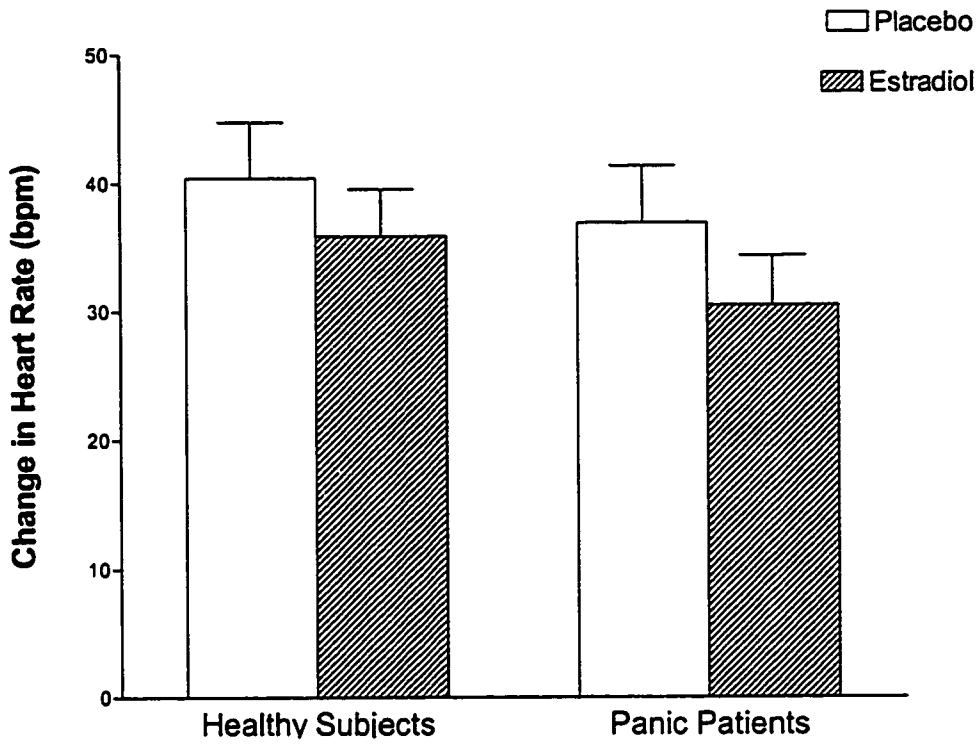

Figure 1: Effects of a 3 day pretreatment of placebo/EE on pentagastrin-induced increase in heart rate (ΔHR). The results are expressed as means ± standard error of means.

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CHAPTER 3

Effect of Medroxyprogesterone Pretreatment on Pentagastrin-Induced Panic Symptoms in Panic Disorder Females*

*The contents of this chapter are represented in a manuscript submitted for publication (J-M Le Mellédo, GS Jhangri, P Lott, GR Tait, K McManus, M Geddes, W Chrapko, N Lara). The author of this thesis played a major role in this study including research visits, data collection, and revision of the manuscript.

3.1. Introduction

There are many reasons to believe that female gonadal hormones influence the course of PD. Indeed, PD is twice as frequent in women as in men, and PD with agoraphobia is three times as frequent in women (Dick et al., 1994; Weissman et al., 1997). Some, but not all studies, have suggested an increase in panic symptomatology during the premenstrum, a phase of the menstrual cycle associated with an abrupt decrease in progesterone levels (see Yonkers et al., 1997 for review). It has also been reported that pregnancy, during which a dramatic increase in both estrogen and progesterone plasma levels take place, is associated with an improvement of the course of PD (Cohen et al. 1994). This phenomenon, however, was not verified in a small pilot prospective study (Cohen et al., 1996) which included severe PD patients treated with antipanic medications. The post-partum period, which is also characterized by a substantial decrease in female hormone levels, seems to elicit or worsen PD (Northcott and Stein, 1994). However, because of methodological difficulties inherent in prospective studies of the natural course of PD, the relationship between hormonal changes and PD has not yet been clearly elucidated. A better understanding of the effects of female hormones in PD is necessary to improve therapeutic management of PD women facing natural or pharmacologically-induced hormonal changes such as choice of oral contraception.

Panic symptoms, similar to the panic symptoms experienced spontaneously, can be induced by pharmacological agents in controlled laboratory situations (Bradwejn et al., 1991; Abelson et al., 1994). These panic challenges are useful tools with which to

study the pathophysiology of PAs. The CCK receptor agonists pentagastrin and CCK-4 are panicogenic agents which induce panic symptoms lasting from two to five minutes.

The anxiolytic activity of progesterone administration has been demonstrated in various animal models of anxiety (Bitran et al., 1993 Bitran et al., 1995; Picazo et al., 1995). ALLO, a metabolite of progesterone with agonist activity at the $GABA_A$/BZD receptor, appears to be responsible for the observed anxiolytic activity of progesterone (Bitran et al., 1993, Bitran et al., 1995, Reddy et al., 1996). Indeed, Bitran et al.. (1993) showed that administration of progesterone is associated with a serum and cortical increase in ALLO and that the anxiolytic activity of progesterone was highly correlated with the increase in ALLO in the brain and blood and was associated with a facilitation of GABA-stimulated Cl^- influx in cortical synaptoneurosomes. They then demonstrated that inhibition of the conversion of progesterone to ALLO, by using a 5- alpha reductase inhibitor, blocks the anxiolytic activity of progesterone (Bitran et al, 1995).

The objective of the current study was to show that pretreatment with medroxyprogesterone acetate (MP), a synthetic progestogen, would decrease the panic response to the panicogenic agent pentagastrin.

3.2. Materials and Methods

3.2.1. Design and Drugs Used

A double blind cross-over placebo-controlled design was used, with randomization of the order of a 3 day pretreatment with placebo and 10 mg of MP (PROVERA®, Upjohn) prior to injections of 30 μg of pentagastrin (PEPTAVLON®, Wyeth-Ayerst,

Philadelphia, PA or Clinalfa, Läufelfingen, Switzerland). The production of pentagastrin by Wyeth-Ayerst was discontinued after the study had begun, so it was necessary to continue the study using pentagastrin from Clinalfa. There was no difference, however, between the effects observed with pentagastrin obtained from one provider versus the other. The purpose of the relatively low dose of pentagastrin was to avoid a ceiling effect and consequently to promote discrimination between the effects of the two pretreatments on the panic response. A relatively low dose of MP was chosen since the purpose was to assess the effect of an average dose of MP used clinically, for example that which is used in birth control pills. It was also sought to avoid MP-induced side effects which would have jeopardized the blindness to pretreatment of the research patients.

The time of intake of pretreatment pills on each of the 3 pretreatment days was scheduled 2 hours before the planned time of the pentagastrin injection which was kept consistent between the two injection visits. In other words, the patients took their pills 50, 26 and 2 h before each pentagastrin injection. The 2 injection visits took place during two consecutive menstrual cycles in the early follicular phase, when endogenous progesterone and estrogens are at their nadir.

3.2.2. Subjects

Twelve women suffering from PD (mean age 32.7 ± 7.6) were recruited by advertisements, but only 9 PD patients completed the study. Subjects were evaluated with the SCID for DSM-IV diagnosis. Patients were physically healthy, not taking psychotropic or hormonal treatment, had regular menstrual cycles and were not abusing

drugs. Any other current diagnosis of axis I disorders was an exclusion criterion applied for the recruitment of the first 8 PD patients. A history of Schizophrenia or Bipolar Disorder was an exclusion criterion. Considering the elevated lifetime prevalence of other psychiatric disorders in patients suffering from PD (Dick et al., 1994), a history of other Axis I diagnoses was not an exclusion criterion provided that their last episode remitted at least 2 years prior to the screening visit. Facing difficulties in recruiting more medication-free panic disorder patients without any current comorbid condition, an additional PD patient was included who also had a current comorbid mild major depressive episode (MDE). The PD was primary and predominant.

Seven out of the 9 women suffering from PD had a history a MDE and 4 out these 6 women also had a history of PTSD. A history of alcohol dependence was found in one woman with a past history of both MDE and PTSD and in one woman with a history of MDE without a history of PTSD. The frequent history of other axis I disorders in our sample underline the difficulties of recruiting PD patients free of medication and without history of any axis I disorder.

3.2.3. Research Visits

Patients received injections of pentagastrin after pretreatment with each of MP or Placebo, in the mid-follicular phase of two consecutive menstrual cycles. Behavioural responses to pentagastrin were evaluated using the PSS scale (Bradwejn et al., 1991) administered once 5 minutes after the pentagastrin injection. Patients rated the intensity of 18 DSM-IV panic symptoms, as a change from baseline, on a 0 (not present)

to 4 (extremely severe) scale (Table 2). A PSS score which is the sum of each individual symptom rating was obtained. The occurrence of PAs following pentagastrin administration was determined based on the DSM-IV criteria for PAs and based on a score of 2 or more on the PSS "anxiety, fear, apprehension" item. During the second injection visit, the patients were also asked to compare their subjective impression of their response to the 2 pentagastrin injections. This was done using a standardized wording ("which injection, according to you, was worse"?) and the question was systematically asked 10 minutes after the second pentagastrin injection.

Subjects arrived at the laboratory between 8:00 and 10:00 AM after an overnight fast. They were seated in a comfortable recliner chair and an i.v. catheter was installed into one of their antecubital veins through which a 0.9% saline solution was slowly dripped. Approximately forty-five minutes after the catheter installation, a 5 s i.v. bolus injection of 30 mg of pentagastrin was administered.

3.2.4. Statistics

Statistical analysis was conducted by using SPSS for Windows®. The two samples t-test adjusting for a period (injection visits) effect for PSS scores and Koch's adaptation of the Wilcoxon-Mann-Whitney rank sum test (Senn , 1993) for each panic symptom were employed to compare the effect between placebo and MP treatment. The t-test was also used to assess whether any injection visits and/or order of pretreatment effect was present in PSS scores. A p-value of < 0.05 was considered significant.

3.3. Results

Three PD women, two randomized to placebo pretreatment first and the other randomized to MP pretreatment first, did not receive the second injection for various reasons. Sequence allocation for the remaining 9 PD patients was the following: 5 women received the first pentagastrin injection after MP pretreatment (sequence: MP/placebo) and 4 women received the first pentagastrin injection after placebo pretreatment (sequence: placebo/MP). Statistical analysis did not show any injection visit effect [$p= 0.24$; $t_7= 1.28$] or order of pretreatment effect [$p= 0.12$; $t_7=1.75$] on the PSS score. None of the patients complained of side effects during the 3 day pretreatment with MP or placebo. However, one woman exhibited breakthrough bleeding, a classical adverse event observed when patients take progestins such as MP, after the second injection prior to which she received MP.

The pentagastrin-induced panic response (PSS scores) obtained for each PD patient after both pretreatment with placebo and MP are shown in figure 2. The results obtained for each panic symptom are presented in table 2. The mean PSS scores (mean $\pm$ standard deviation) following MP pretreatment (28.56 ± 12.80) were significantly decreased when compared to PSS scores following placebo pretreatment (33 ± 14.81) $p=0.048$). When separate analysis was conducted for each panic symptom, statistically significant differences were observed only for anxiety/fear/apprehension, sweating and flushes. Six of the 9 (66.7%) female PD patients found that their response to

pentagastrin was more intense after placebo pretreatment and 2 of 9 (22.2%) found that their panic response was more intense after MP pretreatment while 1 of 9 (11.1%) found no difference. The subjective global impression of the female PD patients was therefore consistent with the observed decrease in PSS scores after MP pretreatment. Panic rate was not different after MP or placebo pretreatment. Seven of the 9 (77.8%) PD patients had a PA with both MP and placebo pretreatment, one (11.2%) had a PA with MP but not with placebo, and one (11.2%) had a PA with placebo but not with MP.

3.3. Discussion

Administration of MP prior to a panic challenge with the panicogenic agent pentagastrin decreased the panic response in female PD patients as assessed with the PSS score and more specifically decreased the anxiety component of the panic response. This effect, although consistent across subjects, was relatively small. Nevertheless, a greater number of PD patients perceived the injection following placebo pretreatment as worse, and this lends clinical relevance to these findings. This effect of MP pretreatment treatment was, however, insufficient to have any significant impact on the panic rate reported by PD patients following pentagastrin injections.

These results are in accord with clinical observations describing improvement of PD at a time when progesterone levels are high, for example during pregnancy, and worsening of PD at times when progesterone levels decrease as during the premenstrual or the post-partum periods. Panic challenge with CO_2 has already been used to indirectly assess the effect of female hormones during the menstrual cycle of PD patients. Perna et

al. (1995) found that the anxiety response to CO_2 was greater during the early-follicular phase (when progesterone levels are low) than in the midluteal phase (when progesterone levels are elevated) in PD patients. The results of this study are, to a certain extent, consistent with the current findings.

The most likely mechanism of this antipanic activity of MP is through its modulation of activity of the GABA system and more particularly the $GABA_A$/benzodiazepine receptor complex. Recent animal studies have shown that the main activity of progesterone on anxiety is mediated through the activity of its metabolite, ALLO at the $GABA_A$ receptor complex. For example, Bitran et al. (1995) have shown, using the elevated plus-maze animal model of anxiety, that the dose-dependent anxiolytic activity of subcutaneous injection of progesterone was correlated with the concentration of ALLO in the cerebral cortex of rats and was associated with an increase of $GABA_A$ receptor function.

There are other possible mechanisms of action which may explain the results of our study. We have shown that the panic response to CCK-4 (another CCK-B receptor agonist) in healthy male volunteers is partially mediated through the beta-adrenergic system (Le Mellédo et al., 1998). On the other hand, Tan et al. (1997) have shown that 10 mg of MP administered during the follicular phase of the menstrual cycle of females suffering from asthma (an illness frequently associated with PD) induced a decreased lymphocyte beta-2-adrenoreceptor density and cyclic-AMP response to isoproterenol. It is therefore possible that part of the antipanic activity of MP observed in our pilot study is mediated through the beta-adrenergic system. This mechanism of action of MP

pretreatment is partially supported by the fact that panic symptoms often associated with increased sympathetic activity, including hot flashes and sweating, were significantly reduced after MP pretreatment.

The small number of patients included in this pilot study is an obvious limitation, but is partially compensated by the within-subjects design. This design was chosen because of the established reliability of the response to CCK_B agonists. Because of the small number of PD patients enrolled in the study, the current results need to be interpreted with caution. The high level of lifetime comorbidity found in this sample of PD patients is in accordance with what has been reported in epidemiological studies and *per se* does not mean that this sample is not representative, although there was a higher level of lifetime history of PTSD in this sample compared to what has been reported in the literature (Magee et al., 1996). Another bias which is inherent in this type of study is that only patients who are ready to have panic symptoms experimentally induced and are willing to delay treatment for a month are studied.

The current results indicate that the interactions between female hormones and anxiety states can be studied experimentally. These results, although limited, are encouraging for the development of further experimental testing of the interactions between progesterone derivatives and anxiety in various paradigms including the progesterone withdrawal paradigm.

Panic Symptoms	Placebo pretreatment	Medroxyprogesterone pretreatment	p Value
Dyspnea	2.56 ± 1.13	2.44 ± 0.73	0.64
Dizziness	1.56 ± 1.24	1.22 ± 1.30	0.510
Unsteadiness	2.44 ± 1.32	2.44 ± 1.13	0.94
Faintness	1.67 ± 1.41	1.44 ± 1.24	0.65
Palpitations/rapid heart	2.44 ±1.51	2.22 ± 1.20	0.48
Trembling/shaking	1.22 ± 1.56	1.44 ± 1.42	0.65
Sweating	1.22 ± 1.39	0.56 ± 0.73	0.02
Choking	1.78 ± 1.48	1.56 ± 1.24	0.41
Nausea	2.00 ±1.58	1.89 ± 1.45	0.90
Abdominal distress	1.67 ± 1.58	1.11 ± 1.54	0.20
Feeling unreal/detached	2.11 ±1.83	1.67 ± 1.73	0.32
Paraesthesia	1.78 ±1.48	1.56 ± 1.51	0.67
Hot flushes/chills	2.00 ± 1.50	1.33 ± 1.41	0.01
Chest pain/discomfort	2.11 ± 1.27	2.00 ± 1.00	0.64
Anxiety/ fear/ Apprehension	3.22 ± 0.67	2.56 ± 0.73	0.003
Fear of dying	0.78 ± 1.56	1.00 ± 1.00	0.12
Fear of losing control	1.56 ± 1.67	1.89 ± 1.17	0.53
Fear of going crazy	0.89 ± 1.76	0.78 ± 1.09	0.64

TABLE 2: Effect of Pretreatment with placebo and medroxyprogesterone on panic symptoms induced by pentagastrin. Symptoms are rated on a 0 to 4 scale. Means and standard deviations are presented. *p values <0.05 are considered statistically significant.

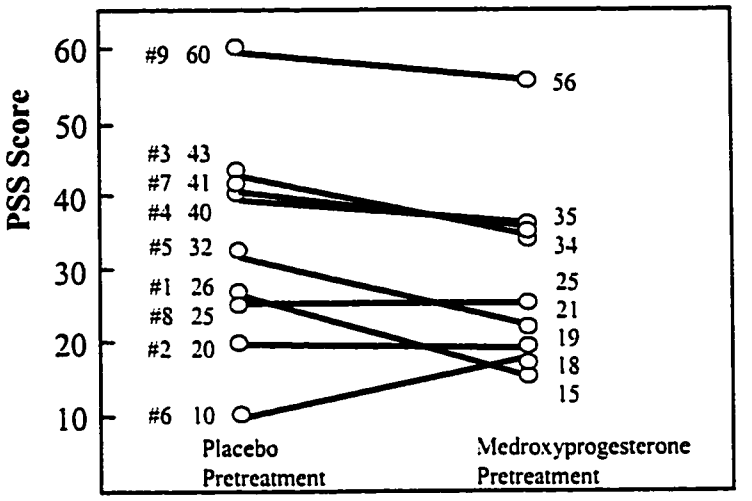

Figure 2: Effect of pretreatment with medroxyprogesterone acetate versus pretreatment with placebo on the panic response to injections of pentagastrin to 9 female PD patients. PSS: panic symptom scale.

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CHAPTER 4

Neuroactive Steroid Levels in Patients with Panic Disorder*

* The contents of this chapter are represented in a manuscript submitted for publication (DR Breitman, GR Tait, K McManus, W Chrapko, Nathalie Lara, J-M Le Mellédo). The author of this thesis played a major role in this study including design and revision of protocol, research visits, data collection, and writing of the manuscript.

4.1 Introduction

PD, an anxiety disorder characterised by spontaneous PAs, affects 1% - 3% of the population (Weissman et al., 1997). The $GABA_A$/BDZ receptor complex has been implicated in the pathophysiology of this disorder. Evidence for its involvement includes dysregulation of $GABA_A$/BDZ receptor binding in patients with PD (Roy-Byrne et al., 1996, Malizia et al., 1998; Bremner et al., 2000), and the clinical effectiveness of BDZs as antipanic agents, through binding at the BDZ site of the $GABA_A$/BDZ receptor. The $GABA_A$/BDZ receptor also modulates the output of many of the systems thought to be involved in anxiety, for instance by abnormal feedback to dopaminergic, noradrenergic, serotonergic, glutamatergic, and CCK systems; and it has been postulated that specific alterations at this site may be implicated in human anxiety disorders (Malizia et al., 1995).

NASs are endogenous progesterone derivatives, including ALLO, pregnenolone sulfate (PREGS), and dehydroepiandrosterone sulfate (DHEAS), which act as allosteric modulators of the $GABA_A$/BDZ receptor complex (Majewska, 1992; Majewska et al., 1986; Paul and Purdy, 1992). There appears to be an elaborate interaction of NAS activity at the $GABA_A$/BDZ receptor complex in the brain (Paul and Purdy, 1992). Evidence exists that ALLO acts as an agonist at the $GABA_A$/BDZ receptor (Majewska et al., 1986) and elicits anxiolytic effects when administered to rodents (Bitran et al., 1991; Wieland et al., 1995). PREGS acts as a mixed agonist/antagonist at the receptor (Majweska and Schwartz, 1987; Majewska, 1992), displaying an anxiogenic response at low doses and an anxiolytic effect at high doses in animal models (Melchior and Ritzmann, 1994). DHEAS has been found to be a noncompetitive antagonist at the

$GABA_A$/BDZ receptor complex (Majewska et al., 1990) and has been shown to induce anxiogenic activity when injected into rodents (Reddy and Kulkarni, 1997).

Levels of ALLO and other NASs in both the brain and plasma of rats increase in response to inhalation of CO_2, a challenge known to induce anxiety-like behaviour in rats and panic attacks in humans. This "stress"-induced release of NAS has been speculated to represent an endogenous homeostatic mechanism for restoring the GABAergic system following stress (Barbaccia et al., 1996b). It is therefore conceivable that PD patients display an impaired ability to release NASs such as ALLO, and that this may manifest as altered baseline levels of these steroids.

To the best of our knowledge, plasma levels of NASs have never been studied in PD, though they have been described in several anxiety disorders. This research team found lower baseline plasma levels of PREGS, relative to HVs, in both GAD patients (Semeniuk et al., 2000) and social phobia patients (Heydari and LeMellédo, unpublished results). Others have found elevated levels of DHEA and DHEAS in PTSD patients (Spivak et al., 2000). Anxiety symptoms and PAs are frequently observed in patients suffering from major depressive disorder (Nutt, 1999). Interestingly, plasma levels of NASs are also altered during a MDE and at least partially normalise following clinical recovery (Ströhle et al., 1999). The aim of this study was to show that baseline plasma levels of ALLO, PREGS, and DHEAS are altered in medication-free PD patients when compared to HVs.

4.2 Materials and Methods

4.2.1. Subjects

Subjects were 16 PD patients (9 male, 7 female) and 16 HVs (9 male, 7 female) matched for age (5 year intervals) and sex. Inclusion criteria for healthy volunteers included no current or lifetime personal history Axis I psychiatric disorder and no family history of PD. PD patients required a diagnosis of PD with or without agoraphobia (DSM-IV criteria). Of the16 PD patients, 11 were diagnosed with "pure" PD (with or without agoraphobia), 2 had comorbid GAD (one male and one female), one male had comorbid social phobia, one male also suffered from GAD and OCD, and another male also displayed comorbid GAD and a specific phobia. All subjects were physically healthy, ages 18-46, and had not used psychotropic medication in the previous 2 weeks (5 weeks for fluoxetine). In addition, subjects were not users of street drugs or alcohol abusers, and were not in current psychotherapy. At the time of plasma collection, all female subjects were in the early follicular phase of their menstrual cycle (day 4 to 8), not taking oral contraceptives in the last 3 months, and not pregnant or lactating. A diagnostic interview including a Structured Clinical Interview for DSM IV (SCID), and a medical assessment were conducted on all subjects. To examine the baseline severity of PD, the Sheehan disability scale (Sheehan et al., 1996) was administered and prospective daily monitoring in the form of a panic diary was completed for a period of one week following the screening visit, measuring frequency of PAs and anticipatory anxiety (Table 3). The Sheehan disability scale, a self-rating scale, is a 3 item scale of impairment, addressing the impact of PD on work (0-10), social life (0-10), and family (0-4). In the panic diary, PAs were evaluated according to DSM-IV criteria. Anticipatory anxiety was rated on a

scale (0-10) and according to what percent of waking hours was spent worrying about PAs.

4.2.2. Blood Sampling

Blood samples were taken from the right antecubital vein.

4.2.3. NAS Analyses

NAS analyses were performed by Dr. R Gupta (PBR Laboratories, Edmonton). For ALLO and PREGS analyses, 2-3 mL of plasma was extracted three times with ethyl acetate (1:1 ratio) and the organic phase was then lyophilised. The residue was dissolved in 1 mL of methanol and 100μL of the prepared sample and was analysed on a Hewlett-Packard HPLC 1100 using a C18 Symmetry column (3.9x150mm, 5μm, Waters Corp., Milford, MA) attached to the guard column (C18, ODS, Octadectyl, 4mmx3mm, Phenomenex, Torrance, CA). The mobile phase varied from 20% HPLC grade acetonitrile (ACN) to 80% ACN delivered at a rate of 1.0 mL/min. The UV monitor was set at 200nm. Authentic samples of the NASs (Sigma, Oakville, Ont.) were used for preparation of calibration curves. Control samples were run along with test samples at regular intervals for consistent reliability of the data through the assay. Concentrations of unknown samples were determined automatically by the Excel® program. The retention time for ALLO was 12.25 minutes at 75% ACN, and that for PREGS was 6.6 minutes at 60% ACN. The coefficient of variation was less than 10% for both ALLO and PREGS. DHEAS levels were quantitated by an enzyme immunoassay. Five calibration points, and two sets of controls were used for analysing the samples. Controls were positioned at regular intervals to maintain the consistency of the data throughout the assay. The samples were incubated with enzyme-labelled antigen (rabbit anti-goat IgG) (Diagnostic

Systems Laboratories Inc., Webster, TX) to compete for their antibody binding sites. The unbound materials were washed off, and the DHEAS concentrations were measured at 450 nm by the hydrolysis of the enzyme-substrate chromogen. The coefficient of variation was 2-4% and the r^2 value was 0.99.

4.2.3. Statistics

The NAS measures between PD patients and HVs were compared as pairs matched for age (5 year intervals) and sex, by two-tailed, paired samples t-tests. Statistical significance was determined based on $p<0.05$. Statistical analysis of the results was performed using SPSS 9.0 for Windows. Results are reported as the mean ± standard deviation (SD).

4.3. Results

Baseline frequency of PAs, anticipatory anxiety, and Sheehan disability scale scores obtained for PD patients are shown in Table 3.

There were no significant differences between HVs and PD patients for: ALLO [(9.01 ± 8.94 ng/mL) vs. (10.69 ± 14.09 ng/mL) ($p=0.667$, $t_{15}=0.424$)], PREGS [(9.69 ± 6.38 ng/mL) vs. (10.02 ± 8.80 ng/mL) ($p=0.888$, $t_{15}=0.144$)], or DHEAS [(2769.25 ± 2125.75 ng/mL) vs. (2238.28 ± 1672.27 ng/mL)($p=0.143$, $t_{15}=1.546$)].

When data were analyzed for male subjects only, there were no significant differences between HVs and PD patients for ALLO [(14.02 ± 8.98 ng/mL) vs. (13.39 ± 17.15 ng/mL) ($p=0.928$, $t_8=0.093$)], PREGS [(12.38 ± 6.83 ng/mL) vs. (14.58 ± 8.95

ng/mL) (p=0.587, t_8=0.565)], or DHEAS [(4168.07 ± 1752.27) vs. (3451.34 ± 1120.97 ng/mL) (p=0.243, t_8=1.261)].

When data were analyzed for female subjects only, there was no significant difference between HVs and PD patients for ALLO [(2.57 ± 2.58 ng/mL) vs. (7.20 ± 8.90 ng/mL) (p=0.215, t_6=1.39)], PREGS [(4.17 ± 4.00 ng/mL) vs. (6.22 ± 3.83 ng/mL) (p=0.337, t_6= 1.044)], or DHEAS [(970.78 ± 703.23 ng/mL) vs. (678.63 ± 520.15 ng/mL) (p=0.400, t_6= 0.906)].

When "pure" PD patients only were compared, and those patients with comorbid diagnoses were excluded from the analysis, there was also no significant difference in ALLO, PREGS, or DHEAS baseline plasma levels between HVs and "pure" PD patients.

4.4. Discussion

There was no significant difference in the baseline plasma levels of ALLO, PREGS or DHEAS between PD patients and HVs. Our negative findings are interesting in light of evidence of altered baseline levels of NASs in patients with GAD (Semeniuk et al., 2000), social phobia (Heydari and Le Mellédo, submitted), and PTSD (Spivak et al., 2000). These results, taken together, suggest that NAS dysregulations are not identical among different anxiety disorders. Our lack of positive findings, however, cannot be explained by the potential confounding due to the comorbidity present in some of the PD patients, since there was also no significant difference in NAS levels between PD patients and HVs when patients displaying comorbid anxiety disorder were excluded. Results for the variables used to assess severity of PD indicate a moderate level of severity. Studying patients with severe PD may yield different results.

Animal studies demonstrate that acute stress results in an increase in levels of some NASs, including ALLO (Barbaccia et al., 1996a; Barbaccia et al., 1996b; Purdy et al., 1991). Although baseline NAS levels were unchanged in the current study, measurements of NASs obtained following stress tests or anxiogenic/panic challenges would be useful, and may uncover dysregulations not apparent in the unchallenged patient. Further research is required to elucidate whether NAS levels elicited in response to acute stress are modified in PD patients as compared to HVs.

Recent studies have demonstrated decreased cerebrospinal fluid (CSF) and plasma levels of NASs in human depression patients, followed by increased levels with clinically effective treatments such as the SSRIs (Romeo et al., 1998; Uzunova et al., 1998; Ströhle et al., 1999), and it has been postulated that this is the mechanism by which these medications act to alleviate anxiety in depression (Uzunova et al., 1998). The results of the current study tend not to support the hypothesis that low levels of NASs observed in depression are related to anxiety; however, this study was limited to PD patients, which may prove inconsistent with states of chronic anxiety.

Although certain NASs such as ALLO can cross the blood-brain barrier (Paul and Purdy 1992), and consequently affect central levels, they are also synthesised *de novo* in the brain (Majewska, 1992). Therefore, since we have measured only peripheral NASs, our results do not preclude dysregulation of NAS activity at the central level.

In summary, baseline peripheral NAS levels (ALLO, PREGS, and DHEAS) are unaltered in patients with moderately severe PD. However, due to the nature of the study, the possibility of abnormal baseline NAS activity at the central level in PD patients and/or dysregulation of the NAS response to stress cannot be excluded.

	Mean	Standard deviation
frequency of panic attacks	4.29	3.43
anticipatory anxiety scale	3.14	3.08
anticipatory anxiety - % time worried about attacks	23.82	19.62
Sheehan – work impairment	3.38	2.25
Sheehan – social impairment	4.06	2.84
Sheehan – family impairment	1.44	1.21

Table 3: Baseline measures of severity of condition, obtained from panic diary entries. Means and standard deviations are presented for the one week period following screening and entering the study.

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CHAPTER 5

Neuroactive Steroid Changes in Response to Challenge with the Panicogenic Agent Pentagastrin*

* The contents of this chapter are represented in a manuscript submitted for publication (GR Tait, K McManus, F Bellavance, Nathalie Lara, W Chrapko, J-M Le Mellédo). The author of this thesis played a major role in this study including design and revision of protocol, research visits, data collection, and writing of the manuscript.

5.1. Introduction

PD, with or without agoraphobia, is an anxiety disorder which affects 1-3% of the general population (Weissman et al., 1997). The hallmark of PD is the repeated sudden occurrence of PAs, episodes characterized by both psychological symptoms (anxiety, fear of dying, fear of losing control, etc.) and physical symptoms (shortness of breath, heart palpitations, chest pain or pressure, etc.) (DSM-IV, American Psychiatric Association 1994).

The $GABA_A$/BDZreceptor complex has been implicated in the pathophysiology of this disorder. Evidence for its involvement includes dysregulation of the $GABA_A$/BDZ receptor in patients with PD (Roy-Byrne et al., 1996; Malizia et al., 1998; Bremner et al., 2000) and the clinical effectiveness of BDZs as anti-panic agents (Chouinard et al., 1982; Primeau and Fontaine 1988). The $GABA_A$/BDZ receptor also modulates the output of many systems thought to be involved in anxiety. For instance, by abnormal feedback to dopaminergic, noradrenergic, serotonergic, glutamatergic, and cholecystokinin systems, it has been postulated that specific alterations at the $GABA_A$/BDZ site may be implicated in human anxiety disorders (Malizia et al., 1995).

NASs are steroidal compounds that exert activity in neural tissue, whether synthesized peripherally or "*de novo*" in the brain (Rupprecht and Hoelsboer, 1999). The NASs ALLO and DHEA, endogenous progesterone derivatives, act at the $GABA_A$/BDZ receptor complex as positive and negative allosteric modulators, respectively (Majewska et al., 1986; Majewska, 1992; Paul and Purdy, 1992). There appears to be an elaborate

interaction between the NASs' activity at the $GABA_A$/BDZ receptor complex and behaviour (Paul and Purdy, 1992). Evidence demonstrates that positive modulators of the $GABA_A$/BDZ receptor, such as ALLO, elicit anxiolytic effects when administered to rodents (Bitran et al., 1991; Wieland et al., 1995). On the contrary, negative modulators of the $GABA_A$/BDZ receptor, such as DHEA, are associated with anxiogenic activity in rodents (Reddy and Kulkarni 1997).

Interestingly, ALLO levels in both the brain and plasma of rats are increased in response to inhalation of CO_2, a challenge known to induce anxiety-like behaviour in rats and PAs in humans (Barbaccia et al., 1996a,b). In Barbaccia et al.'s (1996a) study of male rats, one of the first actions of CO_2 was to induce an increase in binding of t-[^{35}S]-butylbicyclophosphorothionate ([^{35}S]TBPS), a sensitive marker of GABA receptor function (Serra et al. 2000), and this increase was interpreted as a consequence of decreased GABAergic transmission. It was found that inhalation of CO_2 then induced a delayed increase in ALLO levels in the brain and plasma and an increase of DHEA levels in plasma (Barbaccia et al., 1996a).

The current study was interested in assessing whether NASs are released following a panic challenge with a cholecystokinin type B (CCK_B) agonist, pentagastrin. In PD patients, and to a lesser extent in HVs, CCK_B agonists, well-validated panicogenic agents, induce panic symptoms similar to those spontaneously experienced by PD patients, but lasting two to five minutes (Bradwejn et al., 1991a; Bradwejn et al., 1991b; Abelson and Nesse 1994). It was hypothesized that induction of panic symptoms with pentagastrin would result in a release of NASs into plasma. Assuming PD patients and

HVs have an equal ability to release NASs, it was hypothesized that PD patients would display an augmented release of NASs because of their expected greater panic response following pentagastrin challenge.

PD occurs 2-3 times more frequently in females than in males. Fluctuations in female hormones inherent in the menstrual cycle, pregnancy, oral contraceptive use, and menopause have been shown to affect the course of PD (Brier et al., 1986; Cowley and Roy-Byrne 1989; Deci et al., 1992; Ushiroyama et al., 1992; Kaspi et al., 1994). Following the initiation of estrogen-based agents, such as oral contraceptives (Deci et al., 1992; Ushiroyama et al., 1992) and HRT (Price and Heil 1988; Korhonen et al., 1995), some studies report symptomatic improvement of PD and others report worsening. Thus it was further hypothesized that a 3 day pretreatment with ethinyl estradiol (EE) would affect the panic response to pentagastrin and consequently would alter the release of associated NASs.

5.2 Materials and Methods

5.2.1 Design and Drugs Used

A double-blind cross-over placebo-controlled design with randomization of the order of a 3 day pretreatment of placebo (lactose) (Odan Laboratories, Montréal, P.Q. Canada) or EE (Estinyl®) (Schering, Montréal, P.Q., Canada)(50µg/day) was used to assess the effect of a 30 µg 5 second i.v. bolus injection of pentagastrin (Peptavlon®, Wyeth-Ayerst, Philadelphia, PA or Clinalfa, Läufelfingen, Switzerland) on the panic

symptom intensity and the plasma release of the NASs ALLO and DHEA, following each pretreatment. We chose a relatively low dose of EE, since our intent was to assess the effect of an average dose of EE used clinically, for example that which is used in birth control pills. The production of pentagastrin by Wyeth-Ayerst was discontinued after the study had begun, so it was necessary to continue the study using pentagastrin from Clinalfa. However, there was no difference between the effects observed with pentagastrin obtained from one provider versus the other. For each subject, the source of pentagastrin was the same for both injection visits. Informed consent was obtained from each subject and monetary compensation was provided for participation in the study. This study was approved by the Health Research Ethics Board of the University of Alberta, Edmonton, Alberta, Canada.

5.2.2. Subjects

Male PD patients and HVs were targeted through advertisements. Only male subjects were recruited in this preliminary study to avoid the potential confound of fluctuations in NAS related to the menstrual cycle. Of the subjects recruited, 6 (all PD patients) withdrew between the initial screening visit and the first pentagastrin injection visit. One decided not to continue the study upon arrival for the first injection visit, and the other five could not be contacted for rescheduling. Three other PD patients received the first, but not the second, pentagastrin injection. One patient (whose pretreatment was placebo) chose not to continue in the study after the first injection visit. Another patient (whose pretreatment for the first injection was EE) could not be reached for the

rescheduling of his second injection visit. The other patient (whose pretreatment for the first injection was placebo), fainted during the first injection visit so he was withdrawn from the study. Indeed, rare occurences of vasovagal reactions following pentagastrin challenge have been described by other authors (Bradwejn et al, 1992). The subjects who completed the study were 15 male PD patients and 10 male HVs between the ages of 20 and 48 years. The mean age of the PD patients was 32.5 ± 7.6 years while that of HVs was 27.5 ± 8.1 years.

At visit 1 (V1), a diagnostic interview using the Structured Clinical Interview for DSM-IV (SCID) (American Psychiatric Association 1994), was conducted to determine the eligibility of both PD patients and HV. Additionally, an ECG, blood screening, and physical examination of each subject were carried out. To be eligible as an HV, subjects must have had no current or lifetime personal history of an Axis I psychiatric disorder. They were also required to have no family history of PD. Subjects (PD or HV) were excluded from the study if they were heavy smokers (>15 cigarettes/day), heavy coffee drinkers (>5 cups/day), and/or recreational drug users. All subjects were required to be medication-free during the study and PD patients were not to have taken psychotropic medication in the 2 months prior to V1. In addition, they were excluded from participating in the study if medical assessment, blood screening, or ECG indicated they were ill or at risk due to the pentagastrin injection. Eligibility of PD patients, with or without agoraphobia, was defined according to the diagnostic criteria specified in DSM-IV (American Psychiatric Association, 1994). The primary diagnosis required was PD. Because of the within-subjects design and frequent co-morbidity in PD (Katerndahl and

Realini 1997; Kaplan and Sadock 1998), subjects were included in the study even if they had a secondary anxiety disorder such as generalized anxiety disorder GAD, social phobia, specific phobia, OCD, or PTSD. Of the 15 patients who completed the study, 6 had GAD, 3 had social phobia, 1 had specific phobia (fear of heights), 2 had OCD, and 1 had PTSD (related to a motor vehicle accident during adulthood).

5.2.3. Research Visits

At V1, subjects were randomly assigned to receive a 3 day pretreatment with placebo prior to the first pentagastrin challenge at visit 2 (V2) and, a week later, a 3 day pretreatment with EE prior to visit 3 (V3), or vice versa. Each of these two visits (V2 and V3) took place in the morning following 12 hours of fasting. In addition, subjects were not allowed to smoke or have any caffeine the morning of the pentagastrin challenge. They were also told to refrain from alcohol for the 24 hour period prior to 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 chosen to be 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 complained of side effects induced by EE or placebo.

Upon arrival at V2 and V3, subjects were seated in a reclining chair and an i.v. catheter, through which a 0.9% NaCl solution was run at 125 ml/h, was installed in the right antecubital vein. They remained in the semi-supine position for the duration of the procedure. Arrival time and subsequent i.v. installation time (t) were recorded as t=-45

min (45 minutes prior to the time of pentagastrin administration). At t=0 min, pentagastrin was administered as a 30 μg 5 second i.v. bolus injection through a three-way valve connected to the indwelling i.v. catheter. At t=+5 min, the subject's panic symptoms were evaluated using an 18-item DSM-III-R-derived PSS scale (Bradwejn et al 1991a). 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 = -10min, +5min, +15min, +35min, +45min, +90min, blood samples were obtained for the measurement of ALLO and DHEA. Out of 300 samples for each of DHEA and ALLO, 15 samples were missing for DHEA and 13 samples were missing for ALLO due to technical difficulties. All statistical analyses were based on samples obtained, assuming that the missing samples were missing at random.

5.2.4. NAS Analyses

NAS analyses were performed by Dr. R. Gupta (PBR Laboratories, Edmonton). For the analysis of ALLO, 2-3 mL of plasma was extracted 3 times with ethyl acetate (1:1 ratio) and the organic phase was then lyophilized. The residue was dissolved in 1 mL of methanol and 100μL of the prepared sample was analyzed on a Hewlett-Packard HPLC 1100 using a C18 Symmetry column (3.9x150mm, 5μm, Waters Corp., Milford, MA) attached to the guard column (C18, ODS, Octadectyl, 4mmx3mm, Phenomenex, Torrance, CA). The mobile phase varied from 20% HPLC grade acetonitrile (ACN) to 80% ACN delivered at a rate of 1.0 mL/min. The ultraviolet detector setting was 200 nm. Authentic samples of ALLO (Sigma, Oakville, Ont) were

used for preparation of calibration curves. Control samples were run along with the test samples at regular intervals to assure consistent reliability of the data throughout the assay. Concentrations of ALLO in the test samples were determined automatically by IBM's Excel program. The retention time for ALLO was 12.25 min at 75% ACN while the coefficient of variation was less than 10%. For the analysis of DHEA, samples were added to antibody coated microwells (rabbit anti-goat IgG), followed by enzyme-labelled antigen and anti-DHEA serum in a bovine serum albumin-based buffer. The contents were then incubated for 3 hours on an orbital shaker at a speed of 600 rpm at room temperature after which the wells were washed five times with wash solution and dried by blotting on absorbent paper. After the addition of TMB chromogen solution, the wells were incubated at room temperature for 25 min at 600 rpm. The reaction was then stopped by the addition of 0.2 M sulphuric acid and the absorbance was read at 450 nm. The results were then calculated automatically by the Excel® program.

5.2.5 Statistics

One objective was to determine whether pentagastrin induced release of ALLO and DHEA into plasma. Another objective was to determine whether pretreatment with EE, compared to placebo, had any effect on pentagastrin-induced behavioural response (PSS scores) and pentagstrin-induced NAS release. Following the experimental design, a linear model for cross-over design (Jones and Kenward, 1989) was used to analyze the data. The following parameters were included in the model: the main effect of time, the main effect of diagnosis (HVs vs. PD patients), the main effect of pretreatment (EE vs.

Pl), the main effect of visit (V2 or V3), the main effect of order of pretreatment (EE/Pl or Pl/EE), and the following interactions: diagnosis with time, diagnosis with diagnosis with pretreatment, diagnosis with order of pretreatment, diagnosis with visit, 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. *p* values less than 5% were considered significant. Bonferroni's multiple comparisons analyses were also done where appropriate. All statistical analyses were conducted using SAS statistical software for Windows, version 6.12.

5.3. Results

PD patients, compared to HVs, presented with a greater panic symptom severity as indicated by PSS scores (PSS score means ± standard deviations: PD= 35.0 ± 14.7, HV= 22.4 ± 8.9) [F (1, 21) = 9.58; p= 0.0055]; this was not affected by pretreatment [diagnosis by pretreatment interaction; F (1,21) = 0.18; p= 0.6728]. Neither the pretreatment [F(1,21) = 0.20; p= 0.6583], the order of pretreatment (EE/Pl nor Pl/EE) [F (1,21) = 1.24; p= 0.2773], nor the visit (V2 vs. V3) [F (1,21) = 1.33; p= 0.2611] had an effect on the pentagastrin-induced PSS score.

After challenge with pentagastrin, there was a statistically significant release of DHEA over time [F (5,100) = 20.39; p< 0.0001] (Figure 3) which was not affected by pretreatment nor by diagnosis [pretreatment by time interaction F (5, 95) = 0.93; p= 0.4652; diagnosis by time interaction F (5, 100) = 0.62; p= 0.6833; and diagnosis by pretreatment and time interaction F(5, 95)=0.64; p=0.6725] . There was no effect of

pretreatment [F (1,20) = 1.03; p=0.3216] and no effect of visit (V2 vs. V3) [F (1,20) = 0.78; p= 0.3878]. *Post-hoc* analysis with the Bonferroni adjustment for multiple comparisons revealed a statistically significant increase on average in DHEA levels, relative to baseline, at t= +15 min [t_{100}= 5.07; p< 0.001]. A statistically significant decrease in DHEA levels, relative to baseline, was evident at t= +90min [t(100)= -4.60; p<0.001].

Figure 4 shows a trend for a significant release of ALLO. The level of significance for the test of the diagnosis by time interaction is 0.2562 [F(5,100)=1.33] and 0.0833 for the time effect [F(5,100)=2.01]. There was no effect of pretreatment [F(1, 20) = 1.51; p= 0.2332], or visit [F (1,20) = 0.80; p=0.3825].

5.4. Discussion

Compared with HVs, PD patients demonstrated an increased sensitivity to challenge with pentagastrin; this is consistent with other studies where this finding has been discussed extensively (Abelson and Nesse 1994; Van Megen et al., 1994). There was no effect of pretreatment with EE on PSS, confirming preliminary findings published and discussed elsewhere (McManus et al., in press). These findings, however, need to be confirmed in future studies in female patients with PD. The lack of pretreatment effect of EE is in accordance with the lack of consistency in reports on the effect of estrogen-based compounds on panic symptoms in PD patients (Price and Heil 1988; Deci et al.,1992; Ushiroyama et al., 1992; Korhonen et al., 1995).

This is thought to be the first human study to examine the release of NASs with activity at the $GABA_A$/BZD receptor in response to panicogenic challenge. Following pentagastrin-induced panic symptoms, there was a delayed increase in DHEA followed by a decrease, revealing a pattern similar to the first 90 minutes following challenge with CO_2 in rats (Barbaccia et al., 1996a). The animal study suggested a further increase 120 minutes after stress; our time course of blood collection may not have been long enough to detect this.

Further, pentagastrin-induced release of ALLO or DHEA was not affected by pretreatment with EE. The lack of effect of pretreatment was not due to a lack of biological effect of the pretreatment. Indeed, we have found that the same pretreatment with EE increases cortisol response to challenge with pentagastrin (Tait et al., unpublished), indicating that pretreatment with EE had a biological effect different from that of placebo.

The current study only found a statistical trend for a pentagastrin-induced release of ALLO into plasma. Even acknowledging this statistical limitation, the current results suggest a pentagastrin-induced delayed increase of ALLO plasma levels, consistent with findings of a CO_2-induced delayed increase in brain and plasma ALLO levels in rats (Barbaccia et al., 1996a). The current data suggest that extending the time course of blood collection and using a greater dose of pentagastrin may have led to the observation of a definitive and statistically significant time effect for ALLO. Although there was not a statistically significant diagnosis effect or diagnosis by time effect, the graphical results suggest that pentagastrin is released in HVs but not in PD patients.

Given the greater sensitivity to challenge with pentagastrin in PD patients compared to HVs, and assuming that PD patients, relative to HVs, have an equal propensity to release ALLO in response to pentagastrin-induced panic symptoms, PD patients were expected to show a greater release of ALLO following panic challenge, as would be evidenced by a diagnosis by time effect. On the contrary, PD patients seem to have an impaired ability to release ALLO in response to panic challenge with pentagastrin, as was evidenced by a lack of diagnosis by time effect.

It has been proposed that the delayed release of NASs, particularly positive allosteric modulators such as ALLO, may be an endogenous homeostatic mechanism which serves to restore tone to the GABAergic system disrupted by stress (Purdy et al., 1991; Barbaccia et al., 1996a). The suggested decreased ability of PD patients to release ALLO in response to panic symptoms may represent exhaustion of this homeostatic mechanism with consequent progressive deterioration in the $GABA_A$/BZD complex in PD patients (Roy-Byrne et al., 1996; Malizia et al., 1998; Bremner et al., 2000). Interestingly, it has been shown that PD patients, in the months preceding the development of PD, experience significantly more stressful life events such as loss, interpersonal conflicts, and health-related stressors and have a higher proportion of events which are perceived as dangerous, uncontrollable, or undesirable (Finlay-Jones and Brown 1981; Faravelli 1985; Roy-Byrne et al., 1986). It is possible that such substantial stressors induce long-lasting dysregulations of the GABAergic system in vulnerable subjects. The GABAergic system's inability to recover from these long-lasting stressors

may be related to the decreased ability to release ALLO; the clinical correlate of this persisting dysregulation may ultimately be PD.

There are several limitations inherent in the current study. Although it may not be ideal to measure peripheral levels of NASs, there is not, to date, a technique for measuring brain content of NASs in humans. Peripheral changes, however, are informative since ALLO and DHEA both readily cross the blood-brain barrier (Rupprecht and Holsboer, 1999). Clinical findings also support the relevance of measuring peripheral changes in NASs. For example, there is evidence of decreased plasma levels of ALLO in patients suffering from an untreated MDE, with normalization following treatment with the antidepressant fluoxetine, which is also used as an antipanic agent (Ströhle et al., 1999). In addition, dysregulation in plasma levels of NASs in patients with several anxiety disorders has been shown in studies by this research team (Heydari and LeMellédo, submitted; Semeniuk et al., 2000) and others (Spivak et al, 2000).

Another limitation was the time course of blood collection. Because there were no human studies to refer to when designing this study, the time course of blood collection was based on an animal study (Barbaccia et al., 1996a). More specifically, the time course was designed with the intent of detecting the peak release of NASs. Had the time course of blood collection been extended, a prolonged decrease in DHEA may have been observed. Determining the latency for DHEA levels to normalize would be interesting given the literature on the potential role of decreased levels of DHEA in major depressive disorder (Wolkowitz et al., 1999) and the frequent complication of PD with a

MDE (Ballenger, 1998). As well, expanding the time course of blood collection may have led to a definite time effect for ALLO.

The sample of patients in this study may not accurately reflect those with pure PD, due to the comorbidity in some of the patients recruited. In addition, because this study lacked a placebo-controlled injection visit, it is possible that some of the changes in NASs may be due to the experience of injection, as opposed to the pentagastrin-induced symptoms. In future, it will be useful to increase the dose of pentagastrin, introduce a placebo-control injection visit, and expand the time course of blood collection, in a sample of patients with pure PD.

In conclusion, the present study suggested delayed changes in DHEA and ALLO levels in response to panicogenic challenge. The paradoxical absence of a greater pentagastrin-induced release of ALLO in PD patients suggests a potential decreased ability of PD patients to release ALLO in response to a panicogenic agent. Given the evidence suggesting the ability of NASs to alter the $GABA_A$/BZD complex combined with evidence of $GABA_A$/BZD dysregulation in PD, it is crucial to investigate further the role of NASs in PD.

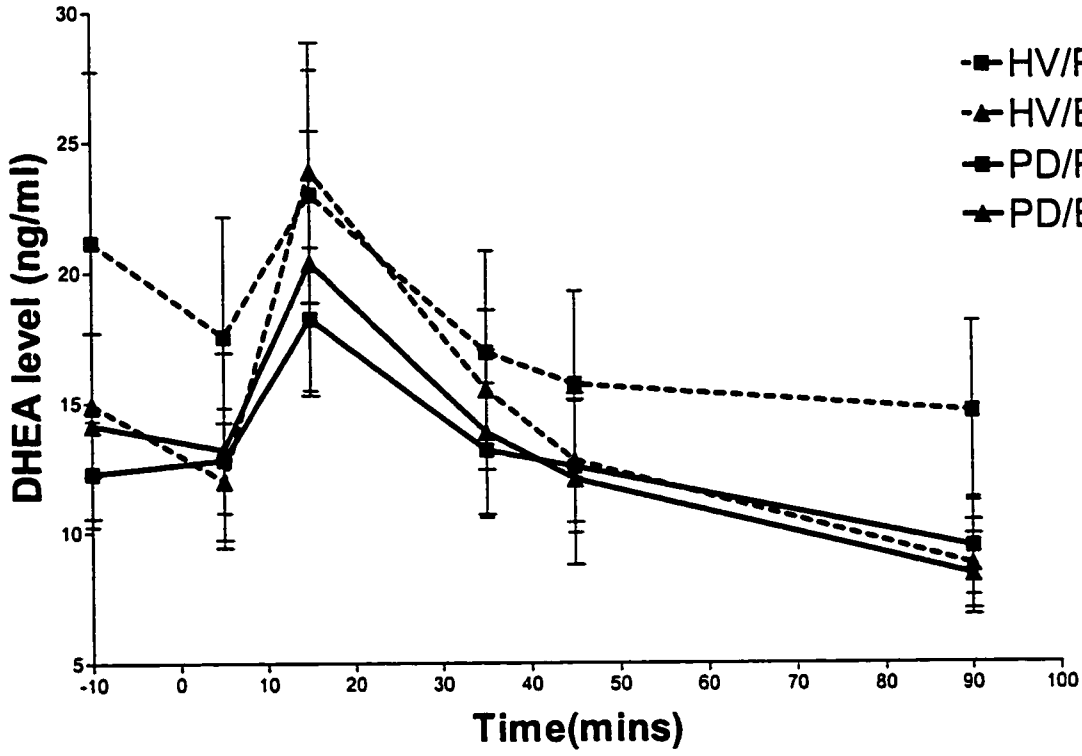

Figure 3: Changes in DHEA levels in response to pentagastrin challenge (HV= healthy volunteers, PD= panic disorder patients, E=ethinyl estradiol pretreatment, P=placebo pretreatment). Graph reflects means ± standard error of means.

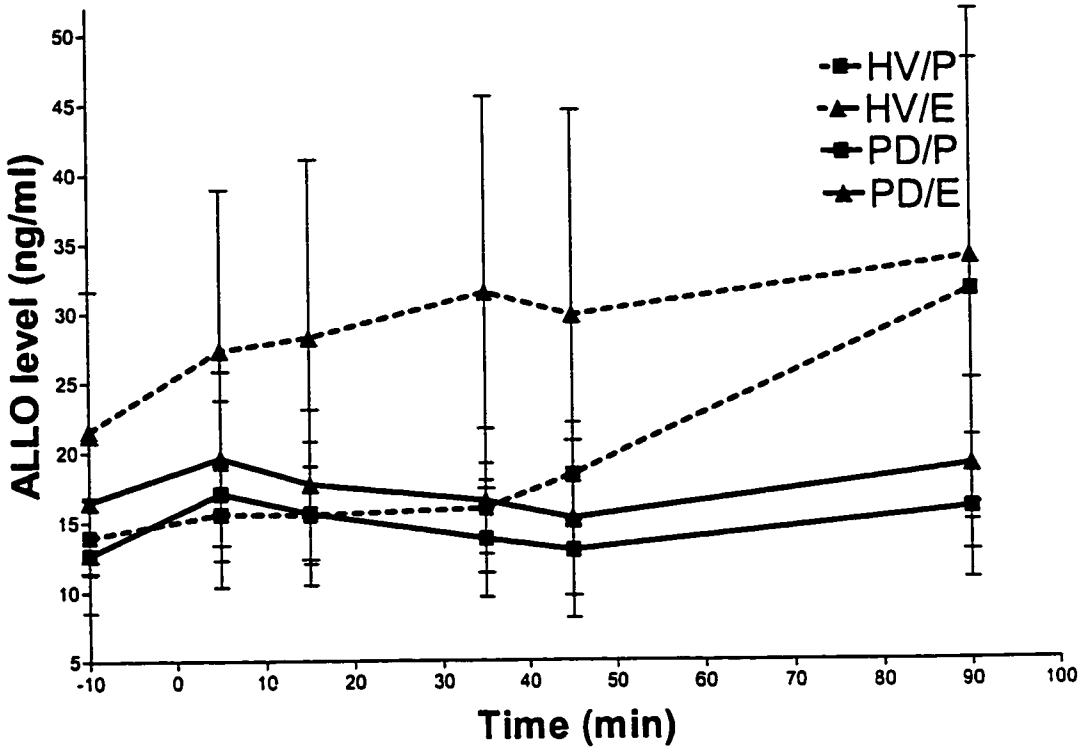

Figure 4: Changes in ALLO levels in response to pentagastrin challenge (HV= healthy volunteers, PD= panic disorder patients, E=ethinyl estradiol pretreatment, P=placebo pretreatment). Graph reflects means ± standard error of means.

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CHAPTER 6

General Discussion and Conclusions

6.1. General Discussion and Conclusions

This thesis examined the role of female hormones and female hormone-derived NASs in PD. In chapter two, it was shown that although exogenous estrogen did not attenuate the pentagastrin-induced behavioral panic response, it did reduce cardiovascular reactivity, probably through actions on the beta-adrenergic system. Knowledge of the potential acute cardioprotective effects of estrogen during PAs is important given the increased risk of cardiovascular death described in PD males; further studies, though, should assess the effect of estrogens on the panic response to pentagastrin in female PD patients.

As was discussed in chapter three, administration of exogenous progesterone attenuated the pentagastrin-induced global behavioral panic response and more specifically the pentagastrin induced anxiety. Though the mechanism of this antipanic activity of progesterone is not known, it is likely mediated through the neuromodulatory activity of its metabolite, ALLO, at the $GABA_A$/BDZ receptor complex. A better understanding of apparent anxiolytic effect of progesterone might be achieved using neuroimaging studies. For example, use of single photon emission computed tomography (SPECT) could measure binding of the BZD antagonist iomazenil to the GABA/BDZ receptor following progesterone pretreatment, relative to placebo pretreatment. As well, nuclear magnetic resonance imagery (NMR) could be used to examine the effect of progesterone levels of $GABA_A$ activity.

Chapter four revealed that PD patients do not differ from HVs at baseline on levels of NASs. This is contrary to findings in investigations of other anxiety disorders by our research team (Semeniuk et al, 2000; Heydari and Le Mellédo, submitted) and others (Spivak et al, 2000). These findings may suggest that dysregulations in NASs may be specific to each anxiety disorder. Though it is more informative to study pathophysiology of a disease in the challenged state, there have not been any other investigations of stress-induced changes in progesterone-derived NASs. The study outlined in chapter five was the first study to examine changes in progesterone-derived NAS levels following panic challenge. PD patients, relative to HVs, appeared to display an impaired ability to release NASs in response to pentagastrin challenge. It is interesting to speculate that it may be, in the case of PD, that these NAS dysregulations exist but are only evidenced when the $GABA_A$/BDZ receptor complex is taxed following acute stress/panic challenge.

Continuing to investigate the role of NASs in the pathophysiology of PD may improve our understanding of the mechanism of antipanic medications. It has been found that the delayed clinical response to antidepressant treatment with SSRIs, also antipanic agents, is associated chronologically with SSRI-induced increases in plasma ALLO (Romeo et al, 1998). As well, SSRIs induce increases in ALLO in the rat brain, mediated through the brain enzyme 3α–hydroxyreductase, which converts ALLO to its metabolite, 5α-dihydroprogesterone (Uzunov et al, 1996; Griffin and Mellon, 1999). A better understanding of NASs and the precise NAS dysregulation in PD may ultimately lead to development of new pharmacological interventions which are hormonally based.

Given the number of people debilitated by PD and its complications, further understanding its pathophysiology and improving its treatment are of paramount importance. This thesis has shown that female hormones can affect both the cardiovascular and behavioural components of the response to panic challenge with pentagastrin. Further, it has suggested alterations in the ability of PD patients to release NASs.

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