Estradiol and progesterone modify the effects of the serotonin reuptake transporter polymorphism on serotonergic responsivity to citalopram

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Abstract

Individual vulnerability to psychopathologies is linked to a number of genetic polymorphisms including the serotonin transporter (5HTT) promoter polymorphic region (5HTTLPR). A single copy of the short variant (s-variant) allele of 5HTTLPR confers increased susceptibility to anxiety disorders and depression and decreased efficacy of serotonin-releasing agents in pharmacotherapy compared to the homozygous long 5HTTLPR variant (l/l). The data suggesting that the 5HTTLPR polymorphism modulates the efficacy of serotonin-releasing agents in pharmacotherapy is inconsistent. Other factors such as age, gender, and hormonal status could interact with 5HTTLPR genotype to affect individual physiological and behavioral responses to serotonin reuptake inhibitors such as citalopram. Indeed, estradiol and progesterone, the primary female steroid hormones, exert an array of effects on the serotonergic system, including 5HTT expression. The present study used ovariectomized female rhesus monkeys to determine the interaction between the 5HTTLPR polymorphism and the effects of mid-follicular levels of estradiol and luteal levels of progesterone on serotonergic responsivity to acute citalopram administration. The increase in serum prolactin, a surrogate measure of serotonin activity, following citalopram administration was significantly larger in l/l females than in s-variant females over the course of two hours during concurrent estradiol and progesterone hormone replacement only. These data suggest that ovarian function and the 5HTTLPR polymorphism interact to gate serotonergic reactivity in females, suggesting that clinicians should be aware of the ovarian status and 5HTTLPR genotype of women when considering serotonergic pharmacotherapy in women.

Keywords

serotonin transporter; citalopram; estradiol; progesterone; females

Introduction

The serotonin transporter protein (5HTT) regulates synaptic concentrations of serotonin, affecting its interaction with the serotonin receptors and a range of behaviors and behavioral and physiological responses (Gingrich & Hen, 2001; Lucki, 1998; Swann, 2003). Dysfunction in the serotonergic system is implicated in an array of psychopathologies, including depression and anxiety (Gingrich & Hen, 2001). Studies in rodents indicate that anxiety- and depressive-like behaviors are modulated by the activity and expression levels of 5HTT, monoamine oxidase and 5HT1A autoreceptor that are critical for regulating serotonin levels and activity (Gingrich & Hen, 2001). Changes in the serotonergic system, evidenced by reduced 5HT1A radioligand binding in individuals suffering from depression (Drevets et al., 1999), are linked to the dysregulation of the stress axis associated with exposure to chronic stressors in rodents (Lopez, Chalmers, Little, & Watson, 1998) and stressful life events in humans (Nemeroff et al., 1984).

Individual vulnerability to affective disorders is associated with genetic polymorphisms in the promoter region of *SCL6A4*, the gene encoding the 5HTT (Caspi et al., 2003). The 5HTT promoter polymorphic region (5HTTLPR) in individuals may consist of two copies of the short allele variant (s/s), two copies of the long allele variant (l/l), or one copy of the short variant and one copy of the long variant (l/s) (Lesch et al., 1996). The short promoter length variant (s-variant) allele in humans, either the homozygous s/s or the heterozygous l/s genotype, has reduced transcriptional activity (Lesch, et al., 1996). Humans carrying this s-variant 5HTTLPR allele have a higher incidence of depression (Caspi, et al., 2003) and anxiety (Lesch, et al., 1996) than individuals having both alleles for the long promoter length variant (l/l).

The efficacy of serotonin-releasing agents for the treatment of psychopathology is also modulated by 5HTTLPR as assessed by changes in peripheral prolactin levels. While an array of neuroendocrine systems, including the stress (Sobrinho, 2003), reproductive (Egli, Leeners, & Kruger, 2010), thyroid (Dare, de Castro, & Maciel, 2008) and immune axes (Grattan & Kokay, 2008), modulate prolactin release, the increase in peripheral prolactin upon administration of serotonin-releasing agents is a valid surrogate measure of serotonin release because serotonin acts to disinhibit prolactin release from dopaminergic control (Fitzgerald & Dinan, 2008) and induces the release of prolactin from the pituitary gland (Matsushita et al., 1983). Individuals with the s-variant allele show diminished prolactin response to clomipramine (Whale, Quested, Laver, Harrison, & Cowen, 2000) and fenfluramine (Reist, Mazzanti, Vu, Tran, & Goldman, 2001) administration. The ability of the 5HTTLPR to modulate the response to treatment with serotonin reuptake inhibitors (SSRIs), such as citalopram, is not consistent in the literature. This inconsistency in SSRI efficacy suggests that factors such as the age, gender, and hormonal status of individuals could influence the effectiveness of these agents to treat psychopathologies (Hoffman, Kaplan, Kinkead, Berga, & Wilson, 2007; Lotrich, Pollock, & Ferrell, 2001; Manuck et al., 2005).

In females, the ovarian steroid estradiol influences a range behaviors and physiological systems (McEwen, 2001; Pfaff et al., 2000; Wallen, 1990). Estradiol works synergistically with luteal phase elevations in progesterone to increase levels of prolactin over the course of the ovarian cycle in female monkeys (Pecins-Thompson & Bethea, 1997; Williams, Gianfortoni, & Hodgen, 1985). The role of estradiol and progesterone in increasing prolactin is also supported by the finding that overall prolactin levels and pulsatility are reduced in postmenopausal women (Katznelson, Riskind, Saxe, & Klibanski, 1998). The effects of estradiol on behavior and physiology are, in part, likely related to its action on the serotonin neural system (Rubinow, Schmidt, & Roca, 1998). Indeed, the serotonin producing neurons

in the raphe are targets of ovarian steroids (Bethea, Lu, Gundlah, & Streicher, 2002; Sheng et al., 2004) and generally act to facilitate serotonin neurotransmission (Bethea, et al., 2002).

Despite the notion that ovarian steroids increase serotonin action, it is unknown how 5HTTLPR polymorphisms affect this response. Understanding how the presence of estradiol and progesterone might affect serotonergic physiology in individuals of each 5HTTLPR genotype is important for the consideration of the pathogenesis and treatment of affective disorders in women. As rhesus monkeys as a species show homologous polymorphisms in 5HTTLPR (Higley et al., 1993; Lesch et al., 1997), the current study was designed to observe the individual and combined effects of estradiol, progesterone, and 5HTTLPR on serotonergic responsivity to the SSRI *s*-citalopram in ovariectomized female rhesus monkeys. We hypothesized that the ability of 5HTTLPR to modulate serotonergic response to SSRI treatment would be dependent on the presence of both estradiol and progesterone.

Materials and Methods

Animals

Subjects were 39 ovariectomized adult (12–17 yr of age) female rhesus monkeys (*Macaca mulatta*) socially housed in indoor-outdoor runs in small social groups (n = 4 or 5 per group) the Yerkes National Primate Research Center Field Station and weighed on average 7.81 ± 0.22 kg. All subjects were genotyped for 5HTTLPR as being homozygous for the long promoter length variant (l/l) or having at least one short promoter length allele (s-variant) (Hoffman, et al., 2007). Animals had access to standard monkey chow (PMI Lab Diets, #5038, St. Louis MO) twice daily, water *ad libitum*, and daily supplementation with seasonal fresh fruit and vegetables. All animals had previously been trained for conscious venipuncture using already validated methods to allow for sample collection without the use of anesthesia (Walker, Gordon, & Wilson, 1982). This procedure allowed for animals to be captured, sampled, and returned to their runs within ten minutes of initial disturbance. The Emory University Animal Care and Use Committee approved all procedures in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals."

Bilateral ovariectomy occurred in all females 20 months prior to initiation of the current study by laparoscopy. Following anesthesia with ketamine and isoflurane, a 1 cm incision was made through the skin, several centimeters proximal to the umbilicus. Two, small cannulas were inserted, one on either side of the lower abdomen for tissue manipulation with instruments and each ovary visualized and isolated with forceps. Vascular clips were then introduced through the other cannula and applied around the ovary. The ovary was cut free and removed through the opening with the forceps and cannula. The same procedure was performed on each side. Females averaged approximately 2.73 ± 0.31 pregnancies prior to ovariectomy. Before the initiation of the current study, these subjects participated in a study assessing how 5HTTLPR modifies the consequences of psychosocial stress on metabolic health (Jarrell et al., 2008) and a study assessing the effects of leptin administration on cortisol secretion (Collura, Hoffman, & Wilson, 2009). Four months elapsed between the cessation of the last study and the initiation of the current study.

As previously described (Jarrell, et al., 2008), the groups of animals used in this study were formed by removing subjects from their natal groups and randomly introducing unfamiliar animals consecutively to form new groups. Briefly, two animals were placed together in two small adjacent indoor-outdoor enclosures and allowed to acclimate to each other for 24 h. A third female was then placed into the adjacent run where she had only visual access to the previously established pair of females. Following 24 h, the third female was introduced to the initial pair. This procedure was repeated until five females were living together in a

single in indoor-outdoor run (Jarrell, et al., 2008). Social groups were formed so that four groups were comprised completely of individuals carrying the l/l 5HTTLPR genotype while four groups were comprised entirely of the s-variant (s/s or s/l) genotype (Jarrell, et al., 2008). Groups were formed 12 months prior to the initiation of the current study and social structure stable since the group formation process.

Treatment Conditions

Animals were studied under four different hormonal conditions each lasting four weeks in duration (Figure 1). The first two and last two treatment periods were separated by a two-week washout. Treatments were counterbalanced across females regardless of social group membership. The four treatment conditions were control (C) followed immediately by estradiol replacement (E2), and progesterone replacement (P4), followed immediately by combination estradiol and progesterone replacement (E2 + P4). The order (C − E2) and (P4 − E2+P4) was randomized among the females but counterbalanced to ensure half the females received the C condition first and the other half the P4 condition first. Hormone replacement was intended to reach mid-follicular levels of estradiol and luteal levels of progesterone whose levels are lower than those observed in women and peak between 4 and 7 ng/ml in female rhesus (Walker, Wilson, & Gordon, 1984; Wallen, Winston, Gaventa, Davis-DaSilva, & Collins, 1984; Wilson, Gordon, & Collins, 1982). Hormone replacement was attained by implanting steroid-filled Silastic capsules subcutaneously between the scapula as previously described at the beginning of each treatment phase to deliver 3 μg/kg/day of E2 and 20 μg/kg/day of P4 for the entire four-week treatment phase (Michopoulos & Wilson, 2011). Selected samples (day 5 and 26) from each of the one-month hormone replacement conditions were assayed to assess serum levels of estradiol and progesterone.

Citalopram challenge to assess serotonin responsivity

During week three of the four-week hormonal replacement, we tested serotonergic responsivity using the highly selective 5HTT reuptake inhibitor, s-citalopram (Hoffman, et al., 2007; Manuck, et al., 2005). We measured serum prolactin levels in response to SSRI administration as a surrogate measure of serotonin responsivity (Matsushita, et al., 1983). The s-citalopram-HCL was first dissolved into a 1% ethanol solution in sesame oil and administered at a dose of 0.55 mg/kg IM (Matsushita, et al., 1983). On the day of the citalopram challenge, a baseline sample was collected at 1300 hr, after which citalopram was administered. Blood samples were then collected at 60 and 120 minutes after injection.

Hormone assays

Serum levels of prolactin were measured by RIA kit from Diagnostic Systems Laboratories (DSL, Webster, TX) with a sensitivity of 0.10 ng/ml and an inter- and intra-assay CV of 6.3% and 9.1%, respectively (Hoffman, et al., 2007). Verification of the efficacy of estradiol Silastic capsules was determined by assaying selected samples using a modification of a previously validated estradiol assay from Diagnostics Products Corporation (DPC, Los Angeles, CA) (Pazol, Kaplan, Abbott, & Wilson, 2004). Using 200 μl of serum, the assay has a sensitivity of 5 pg/ml and an intra- and inter-assay coefficient of variation (CV) of 5.2% and 11.1%, respectively. Efficacy of Silastic capsules containing progesterone was determined using a modification to a previously described assay using a commercially available kit (DPC) (Wilson et al., 2003). Briefly, 125 μl of sample were extracted with anesthesia grade ether and the organic layer evaporated off by a stream of N2. The sample was reconstituted in 125 μl of the assay buffer and replicates were assayed following the kit protocol. The sensitivity of the assay is 0.10 ng/ml with an inter- and intra-assay CV of 8.14% and 7.73%, respectively. All assays were conducted in the Biomarkers Core Lab at the Yerkes National Primate Research Center.

Statistical analyses

The main effects of the between-subject factor 5HTTLPR genotype (l/l vs. s-variant), and the within-subject factors of hormonal treatment (C vs. E2 vs. P4 vs. E2 + P4) and time (baseline, 60 min and 120 min following citalopram administration), as well as their interactions were analyzed with analysis of variance for repeated measures. A test result with a p ≤ 0.05 was considered significant and post hoc analysis undertaken by the Newman-Keuls test to isolate specific group or treatment effects. Data are summarized as mean ± standard error of the mean (SEM).

Results

Hormone Replacement

Hormonal treatment of estradiol and progesterone yielded comparable levels of hormones in all females, regardless of 5HTT (Figure 2; p > 0.05). Analysis of selected samples (day 5 and 26) during E2 versus no E2 replacement showed hormone replacement achieved mid-follicular phase levels of E2 (26.0 ± 1.92 vs. <5.0 pg/ml). Similar analysis of P4 replacement versus no P4 replacement showed luteal phase levels of progesterone were achieved by the treatment (4.29 ± 0.11 vs. 0.61 ± 0.03 ng/ml).

Prolactin response to citalopram administration

Baseline levels of prolactin immediately preceding citalopram administration (1300 hr) were significantly increased during the combination E2 + P4 treatment compared to all other treatments (Figure 3; $F_{1, 35} = 13.6$, p=0.001). Levels of prolactin increased over time ($F_{1, 35} = 32.3$, p<0.001), with the highest levels occurring at 120 mins following citalopram injection (p<0.001). This main effect of time interacted significantly with hormonal treatment (Figure 3; $F_{1, 35} = 7.79$, p<0.001). Prolactin levels were increased the highest after combination E2 + P4 treatment at baseline (p<0.001), 60 (p=0.001) and 120 mins (p<0.001) following citalopram injection (Figure 3). Additionally, treatment with P4 alone resulted in the lowest levels of prolactin by 120 minutes compared to all other treatments (p=0.028; Figure 3).

Even though there was no main effect of 5HTTLPR genotype on overall prolactin levels (p > 0.05), 5HTTLPR genotype interacted significantly with both time and treatment ($F_{1, 35} = 2.45$, p=0.026). Females with the l/l 5HTTLPR genotype responded to the combined E2 + P4 treatment with incremental increases in prolactin following citalopram administration at both 60 (p=0.029) and 120 (p=0.018) mins that were significantly greater than those seen in s-variant females (Figure 3). However, baseline levels of prolactin were increased in both l/l and s-variant females during combined E2 + P4 administration compared to all other treatments (p=0.255; Figure 3).

Discussion

Our data indicate that the hormonal modulation of serotonin release is mediated by the 5HTTLPR, as indicated by the increase in serum prolactin levels, as a surrogate measure of serotonin release (Matsushita, et al., 1983), in response to citalopram administration. Previous studies in humans and rhesus monkeys show that individuals with the s-variant 5HTTLPR allele have diminished response to the SSRIs clomipramine and citalopram (Hoffman, et al., 2007; Whale, et al., 2000). In our study we show that this effect is dependent upon the concurrent treatment with physiological concentrations of estradiol and progesterone, as the increase in prolactin levels following citalopram administration was greater in l/l females than in s-variant females during concurrent estradiol and progesterone hormone replacement. These data suggest that accounting for ovarian hormone levels and

5HTTLPR could help determine the efficacy of pharmacotherapy with serotonin-releasing agents in women.

The inconsistency in the effectiveness of SSRIs in the treatment of affective disorders, including depression, is linked to individual genetic variability, including the 5HTTLPR (Lotrich, Pollock, Kirshner, Ferrell, & Reynolds Iii, 2008). Physiologically, s-variant individuals show diminished prolactin response to citalopram compared to l/l individuals, and functionally show decreased activation of cortical regions than l/l individuals after citalopram administration as assessed by changes in cerebral glucose metabolism (Smith et al., 2004). Differences in responsivity to serotonin-releasing agents could be due to the fact that the s-variant 5HTTLPR allele not only confers reduced transcription and thus decreased 5HTT expression (Smith, et al., 2004), but also lowers the efficacy of 5HTT transport (Heils, Mossner, & Lesch, 1997). Mouse models of 5HTT deficiency that decrease the amount of serotonin reuptake, similar to that characteristic of the s-variant 5HTTLPR, show altered patterns of serotonin receptor levels (Fabre et al., 2000) similar to changes seen in individuals suffering from depression (Drevets, et al., 1999).

However, many of these studies assessing the role of 5HTTLPR in mediating individual vulnerability to affective disorders and altered response to pharmacotherapies do not control for gender or take gonadal hormones into account, a surprising notion considering that susceptibility to affective disorders favors women over men, two to one (Weissman & Olfson, 1995). Indeed, the results from the current study suggest that the inconstancies concerning 5HTTLPR's ability to influence physiological response to SSRI administration (Kraft et al., 2007; Lotrich, et al., 2001; Murphy, Hollander, Rodrigues, Kremer, & Schatzberg, 2004) might stem from gender and hormone level differences as the ability of 5HTTLPR to confer differential response to citalopram administration in female monkeys was dependent on the presence of both estradiol and progesterone. Indeed, estradiol is capable of modulating 5HTT mRNA and protein levels, even though the effects of estradiol on 5HTT levels are contradictory. Acute estradiol exposure in rodents increases 5HTT mRNA and protein levels (McQueen, Wilson, Sumner, & Fink, 1999; Sumner et al., 1999; Sumner et al., 2007) whereas chronic estradiol administration decreases 5HTT mRNA levels in monkeys (Pecins-Thompson, Brown, & Bethea, 1998), suggesting that dosing of estradiol, time of estradiol exposure and species differences influence 5HTT levels. Additionally, *in vitro* studies have shown that estradiol treatment decreases the reuptake of serotonin, thus increasing serotonin neurotransmission (Koldzic-Zivanovic, Seitz, Watson, Cunningham, & Thomas, 2004).

While the finding that 5HTTLPR modifies the efficacy of estradiol and progesterone on serotonergic activity is novel, the notion that concurrent estradiol and progesterone maximizes prolactin, and thus serotonin, response to a serotonin-releasing agent is not. Estrogen primed macaque females injected with progesterone to mimic the ovarian cycle changes in hormone milieu results in an increase in prolactin release (Pecins-Thompson & Bethea, 1997; Williams, et al., 1985). Mechanistically, these hormones act synergistically to decrease levels of the serotonin metabolite, 5HIAA (5 hydroxyindole acetic acid), and alter gene and protein expression of key mediators in serotonin function in a manner that suggests increased serotonin activity (Bethea, et al., 2002). A microdialysis study confirmed that serotonin levels are increased 40 hours following progesterone administration on an estradiol background in macaque females, implicating a role for progesterone receptor activation (Centeno et al., 2007). The role of progesterone's actions on serotonin release via its hormone receptor is strengthened by data describing that expression of progesterone receptors is upregulated by estradiol (Romano, Krust, & Pfaff, 1989) and increases in serotonin are seen only when estradiol and progesterone are co-administered (Centeno, et al., 2007).

Progesterone administration by itself increased citalopram-induced prolactin levels the least in all females compared to all other treatments including control. The absence of estradiol and the fact that the effect of progesterone by itself is different than that in the combined E2 + P4 treatment suggest that the facilitating effect of progesterone on serotonin may be dependent upon the presence of estradiol and its ability to upregulate progesterone receptors (Romano, et al., 1989). The minimal increase in serotonin response to citalopram associated with P4 alone could be due to the actions of its metabolite, allopregnanolone, as estradiol is necessary for the upregulation of progesterone receptors (Romano, et al., 1989). Allopregnanolone acts centrally as a potent modulator of γ-aminobutyric acid (GABA) type-A receptors, increasing GABA-induced inhibitory current (Majewska, Harrison, Schwartz, Barker, & Paul, 1986; Twyman & Macdonald, 1992). The synthesis of allopregnanolone occurs in structures that produce and are innervated by serotonin, such as the central nucleus of the amygdala, hippocampus, and dorsal raphe nucleus, all areas critical to affective behavior (Agis-Balboa et al., 2006; Stoffel-Wagner, 2003). This pathway presents a mechanism by which progesterone, via allopregnanolone, decreases serotonin and prolactin response to citalopram administration by inhibiting serotonergic neurons from releasing serotonin (Gao, Fritschy, Benke, & Mohler, 1993).

While ovarian steroid hormones have a pronounced effect on the serotonergic system and indeed modulate the release of prolactin from the pituitary gland, there are many other neuroendocrine systems that affect prolactin levels, including the stress system (Sobrinho, 2003). For this reason, and the fact that measuring prolactin response to a serotonin-releasing agent is a surrogate measure for central serotonin release (Reist, et al., 2001; Whale, et al., 2000), our results should be considered preliminary as further investigation must be undertaken to assess how estradiol, progesterone and 5HTTLPR influence central changes in serotonergic activity in female rhesus monkeys. Furthermore, the current study only assessed acute physiological responses to an acute SSRI injection, without assessing coincident short-term behavioral changes. A study looking at the effects of estradiol and progesterone and chronic SSRI administration on physiology and behavior is warranted to provide more translational validity to the current data set. Finally, it is important to note that the current study was undertaken in hormone replaced, ovariectomized females, providing a more similar context for postmenopausal women receiving hormone replacement therapy than premenopausal cycling women.

In conclusion, the current findings corroborate our previous account of diminished serotonin response to citalopram, inferred from changes in prolactin, in gonadally intact s-variant females (Hoffman, et al., 2007) and extend these data by showing that this effect of 5HTTLPR is most evident when both ovarian steroid hormones, estradiol and progesterone, are restored to follicular and luteal phase concentrations, respectively. Importantly, these data suggest that clinicians should be aware of ovarian status in patients being considered for treatment with serotoninergic agents, as the hypogonadism associated with chronic psychosocial stressors in women (Berga et al., 1989) might decrease responsiveness to SSRI treatment. It is also possible that accounting for hormones in pre-, peri-, and post-menopausal women could explain some of the variability surrounding the efficacy of SSRIs in responders and non-responders to pharmacotherapy in women. Based on our findings we would predict that l/l 5HTTLPR females with endogenous estradiol and progesterone activity would be the most likely to respond to SSRI treatment. Taken together, these data from female rhesus monkeys indicate that determining hormonal milieu and 5HTTLPR genotype in women suffering from affective disorders is an important consideration for clinicians when considering pharmacotherapy targeting the serotonin system (Lotrich, et al., 2001).

Acknowledgments

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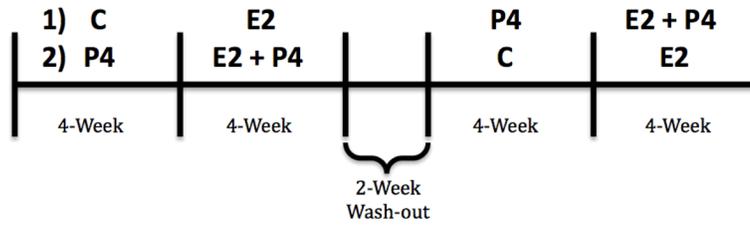
Figure 1.
Illustration of the sequence and duration of treatment conditions. Animals were studied under four treatment conditions: control (C) followed immediately by estradiol replacement (E2), and progesterone replacement (P4), followed immediately by combination estradiol and progesterone replacement (E2 + P4). The first two and last two treatment periods were separated by a two-week washout. The order (C − E2) and (P4 − E2+P4) was randomized among the females but counterbalanced to ensure (1) half the females received the C condition first and (2) the other half the P4 condition first.
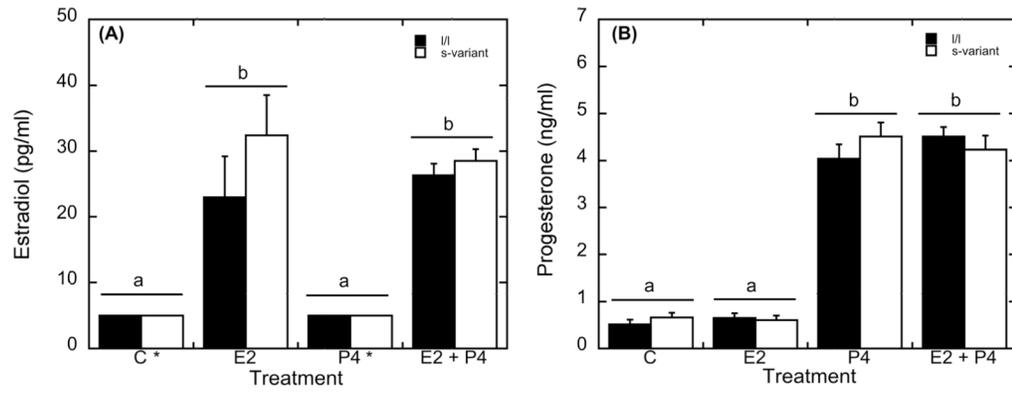
Figure 2.
Mean ± SEM levels of (A) estradiol (E2; pg/ml) and (B) progesterone (P4; ng/ml) upon hormonal replacement for each phase of the study broken down by 5HTTLPR genotype. Replacement of E2 + P4 during study was successful in elevating hormones to comparable levels in all females, regardless of 5HTTLPR genotype ($p > 0.05$). Letters note significant increases in hormone levels. Asterisks (*) denote that E2 levels during the control (C) and progesterone (P4) conditions were less than 5.00 pg/ml.
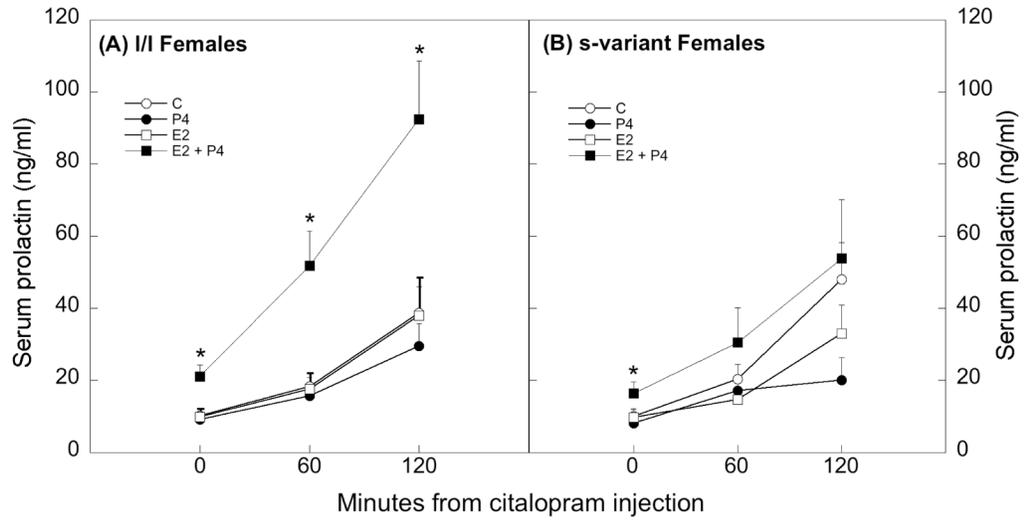
Figure 3.
Mean ± SEM prolactin levels following at zero, 60 and 120 following citalopram injection in females with l/l (A) or s-variant (B) genotype of 5HTTLPR. Asterisks in (A) depict significant increases in prolactin levels at all time points, and in (B) denote a baseline difference in prolactin levels.