Psychopharmacology (2004) 175:247–255
DOI 10.1007/s00213-004-1811-0

ORIGINAL INVESTIGATION

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Antidepressants, sex steroids and pituitary–adrenal response in sheep

Received: 1 December 2003 / Accepted: 15 January 2004 / Published online: 3 March 2004

The importance of sex differences in major affective diseases such as depression is providing a new focus for investigating the interactions between sex, sex steroids and antidepressants.

In this study, we examined the acute effects of sertraline, a selective serotonin reuptake inhibitor (SSRI) and imipramine, a tricyclic antidepressant (TCA) on the endocrine endpoints, adrenocorticotropin (ACTH) and cortisol secretion in gonadectomised male and female sheep.

Each sheep was treated with an acute subcutaneous (SC) injection containing vehicle, sertraline (5 and 10 mg/kg), or imipramine (10 mg/kg) in the presence and absence of sex steroid replacement. In males, SSRI treatment consisted of testosterone (2×200 mg SC pellets), and in females, estradiol (1 cm SC implant) plus an intravaginal controlled internal drug release device containing 0.3 g progesterone. ACTH and cortisol were measured in jugular blood.

Female sheep responded to sertraline treatment with dose-dependent ACTH and cortisol increases that were unchanged by sex steroid replacement. In castrated males, however, only the highest dose of sertraline increased ACTH and cortisol, and this increase was abolished in the presence of testosterone replacement. Imipramine affected neither ACTH nor cortisol secretion in either the sex or sex steroid condition.

We conclude that the sex and sex steroid-related differences in the male and female responses to sertraline treatment may reflect sex and sex steroid dependent differences in serotonergic activation of the HPA axis. This highlights the potential significance of sex and circulating sex steroids in modulating neuroendocrine responses to antidepressants, and may have an impact on our understanding of the actions of these drugs in men and women.

Keywords Antidepressant · Sertraline · Adrenocorticotropin · Cortisol · Sex steroids · Testosterone · Oestradiol · Progesterone · Gonadectomy · Sheep

Introduction

Mental disorders such as depression are a serious public health problem. The World Health Organization (Dec 2001) estimates that 121 million people worldwide currently suffer from depression, with approximately 5.8% of men and 9.5% of women experiencing a depressive episode in any given year (http://www.who.int/inf-fs/en/fact265.html/ Fact sheet No. 265, December 2001). A contributing factor to the predominance of this disease in women may be the dramatic hormonal changes that take place during a woman's reproductive years (Steiner et al. 2003). The critical times at which depression is more likely to be diagnosed include menarche, the postpartum stage following pregnancy and during perimenopause (Garvey et al. 1983; Parry 1989), for which a decline in oestrogen and progesterone levels is thought to be responsible (Gitlin and Pasnau 1989). Oestradiol replacement therapy has been shown to improve mood symptoms of depression or anxiety in women (Soares et al. 2003). Changing sex steroid levels may also affect mood in men, as changes in mood occur in association with a decline in free plasma

Parts of this work were presented at the meeting of the Endocrinology Society, Philadelphia, Pa., USA in June 2003, and at the annual meeting of the Endocrine Society of Australia, Melbourne, September, 2003.

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testosterone levels in ageing men (Vermeulen 1993). In addition, testosterone replacement therapy has been reported to improve mood in hypogonadal men (Wang et al. 1996).

The link between depression and hypothalamic–pituitary–adrenal (HPA) dysregulation is a speculative one. The coincidence of depression and dysregulation of the HPA axis in both melancholic (Ehlert et al. 2001) and atypical depression (Gold et al. 1995) may indicate a common aetiology. Antidepressants have been shown to normalize the activity of the HPA axis, by increasing glucocorticoid receptor (GR) mRNA expression (Pepin et al. 1989), up-regulating GR number (Reul et al. 1993), as well as suppressing corticotropin releasing hormone (CRH) synthesis (Michelson et al. 1997). The efficiency of glucocorticoid signaling is increased, thereby correcting the impaired negative feedback that is thought to be the primary cause of the hyperactivity of the HPA axis in melancholic depression (Young et al. 1995).

Sex differences in the function of the HPA axis may be due to sex differences in the levels of gonadal hormones such as oestrogen, testosterone and progesterone. Androgens and oestrogen can act at each level of the HPA axis, affecting glucocorticoid synthesis, as well as CRH, arginine vasopressin (AVP), ACTH and glucocorticoid release (Canny et al. 1999). In the rat, it has been proposed that oestrogen enhances HPA axis responses, whereas androgens depress HPA axis activity (Viau et al. 2001; McCormick et al. 2002). In the sheep, the acute administration of different stressors to intact and gonadectomised rams and ewes resulted in sex differences in the cortisol responses that were stressor-dependent but were not affected by gonadal status (Turner et al. 2002). In addition, the ACTH and cortisol responses of intact and ovariectomized ewes to various acute stressors were attenuated during the follicular phase of the oestrous cycle in the intact ewes, as well as following the administration of high physiological doses of oestradiol in the ovariectomized ewes (Komesaroff et al. 1998). In contrast, studies in wethers or castrated rams suggest that testosterone treatment has little effect on either basal, stress-induced or CRH and AVP-induced increases in cortisol secretion (Arnold et al. 1996; Tilbrook et al. 1999a,b).

Imipramine is a tricyclic antidepressant (TCA) prescribed in the treatment of major depressive disorders (Tamayo et al. 1992). The primary actions of imipramine and its active metabolite, desipramine, are to inhibit the reuptake of both serotonin (5-HT) and noradrenaline (NA) from the synaptic cleft. Sertraline is a selective serotonin reuptake inhibitor (SSRI) that potently and selectively inhibits the reuptake of 5-HT. Moreover, sertraline has minimal effects on neurotransmitters such as the central NA and dopaminergic systems (Hiemke and Hartter 2000). Sex differences have been reported in the antidepressant efficacy of sertraline and imipramine in men and women (Kornstein et al. 2000; Baca et al. 2004), although this finding may only apply for some (Martenyi et al. 2001) but not all TCA and SSRI antidepressants (e.g. Hildebrandt et al. 2003). In addition, there remains a paucity of data regarding sex differences in the effects of antidepressants on other endpoints, such as the HPA axis. In a recent study, it was found that acute administration of sertraline to gonadectomised ewes (without sex hormone replacement) increased plasma cortisol and ACTH concentrations in a dose-dependent manner (Broadbear et al. 2004). This effect was not seen in castrated rams. The purpose of the present study was to determine whether this difference would persist in the presence of the sex steroids. The hypothesis under investigation is that sex and sex steroids will differentially influence the endocrine responses to exogenously administered antidepressant agents that modulate 5-HT signaling. More specifically, we predict that the presence of mid-luteal levels of oestrogen and progesterone will enhance HPA response, whereas testosterone will be without effect on the HPA response to antidepressant treatment.

Materials and methods

All experiments were conducted at the Prince Henry's Institute of Medical Research (PHIMR) at the Victorian Institute of Animal Science, situated at Werribee, Victoria, Australia.

Animals and ethical clearances

Six castrated rams and six ovariectomized ewes of the Romney Marsh breed were used in this study. The animals were gonadectomised as sexually mature adults at least 3 months prior to the experiment, and ranged in live weight from 63.5 to 79.5 kg.

Animal experiments used in this study conformed with the "Principles of laboratory animal care" (http://www.nap.edu/readingroom/books/labrats/) and were approved by the Animal Experimentation Ethics Committees of the Victorian Institute of Animal Science and the Department of Physiology, Monash University. Experimental protocols conformed with the requirements stipulated by the Australian Prevention of Cruelty to Animals Act 1986 and the NH&MRC/CSIRO/ARC/AVCC "Code of Practice for the Care and Use of Animals for Scientific Purposes."

Study design

A crossover design was used, with half of the subjects receiving sex steroid replacement during the two arms of the study. During the first, three castrated rams and three ovariectomized ewes were randomly assigned to receive sex steroid replacement. Testosterone replacement in rams consisted of the subcutaneous (SC) placement of two testosterone implants, both of which contained 200 mg testosterone (Organon, Australia). Three OVX ewes were treated with progesterone (Van Cleeff et al. 1998) and oestradiol (Evans et al. 1994) to simulate luteal phase levels of these hormones. Oestradiol was administered SC via a 1 cm implant, and progesterone replacement was by means of an intravaginal controlled internal drug release device (CIDR, Riverina Artificial Breeders Pty Ltd, Albury, NSW, Australia) containing 0.3 g progesterone. The CIDRs were replaced on a weekly basis. Sex steroid replacement commenced 2 weeks prior to the first experiment.

In week 3, sheep received sham treatment, which involved blood sampling but no injection. This was done primarily to habituate the animals to the experimental procedure. During weeks 4–7, sheep were randomly assigned to receive a single SC injection of 10 mg/kg imipramine (purchased from Sigma-Aldrich, St Louis, Mo., USA), 5 or 10 mg/kg sertraline hydrochloride (Pfizer Pty

Limited, Groton, Conn., USA), or vehicle (50% dimethyl sulphoxide, 50% saline), with an injection volume of 0.2 ml/kg. Experiments were conducted at weekly intervals. At the end of week 7, the sex steroid implants were removed from the first six sheep. After a 1-week break, sex steroids were implanted in the remaining six sheep and the testing procedure from the first arm was then repeated.

On the day prior to blood sampling, the sheep were taken from pasture into a well-ventilated, naturally lit shed and placed into single pens. Although they were housed individually, the sheep were in close proximity to one another, and had audio-visual contact during the experimental periods. The animals were fed with chaffed lucerne hay at 0830 hours and had uninterrupted access to water. One jugular vein was catheterized with a 12-gauge catheter (Dwellcath; Tuta Laboratories, Australia) that was secured to the skin with nylon suture. A local anaesthetic (xylocaine; Astra Pharmaceuticals, Australia) was administered at the incision site prior to cannulation. A polyethylene manometer line was connected to the catheter, closed with a three-way tap and then attached to the rear of the pen. This enabled blood sampling of the animals to be done remotely. Heparinized (100 IU/ml) normal saline was flushed through the line twice daily.

Blood sampling

Blood samples (7 ml) were collected for 4 h at 10-min intervals via the jugular catheter. The drug or vehicle treatment was administered (SC) 2 h after commencement of blood collection. Blood was transferred to tubes containing 250 μl of ACTH degradation inhibitor mix (0.2 M *n*-ethyl-maleimide; 50 mM ethylene diamine tetra-acetic acid (EDTA disodium salt); and 2100 IU/ml aprotonin in saline). Blood samples were centrifuged at 3000 rpm for 10 min at 4°C and plasma was harvested and stored at −20°C for subsequent ACTH and cortisol assay.

Radioimmunoassays

ACTH. ACTH was measured in plasma sampled during the hour prior to treatment, and the hour post-treatment. ACTH was first extracted from plasma using 20 mg silicic acid (diameter <20 μm; Aldrich Chemical Company, Milwaukee, Wisc., USA) and following the method described by Rees et al. (1971). ACTH was reconstituted in buffer and assayed following the procedure published by Canny et al. (1999). The sensitivity of the assay was approximately 2 pg $ACTH_{1–39}$/tube. All samples were assayed in duplicate. The intra-assay coefficients of variation were 10.3% (n=16 replicates of a single sample with a mean concentration of 17.1 pg/ml) and 7.8% (n=19 replicates of a single sample with a mean concentration of 702 pg/ml). The inter-assay coefficients of variation were 13.1% (n=10 replicates of a single sample with a mean concentration of 16.2 pg/ml) and 11.0% (n=8 replicates of a single sample with a mean concentration of 686 pg/ml). Recovery (mean±SD) of ACTH following extraction was 70±15.5%. Individual hormone concentrations were not corrected for efficiency of extraction.

Cortisol. Blood samples from all time points were assayed to determine plasma cortisol concentrations in animals from all treatment conditions. The assay procedure that was used to measure plasma cortisol levels has been published previously (Broadbear et al. 2004). The sensitivity of the assay was 0.2 ng/ml. The intra-assay coefficients of variation were 16.3% (n=16 replicates of a single sample with a mean concentration of 12.6 ng/ml) and 7.8% (n=19 replicates of a single sample with a mean concentration of 73.6 ng/ml). The inter-assay coefficients of variation were 14.7% (n=18 replicates of a single sample with a mean concentration of 11.1 ng/ml) and 11.8% (n=14 replicates of a single sample with a mean concentration of 69.0 ng/ml).

Testosterone and progesterone. Single samples, taken approximately 30 min prior to drug or vehicle treatment each week from both steroid-treated and untreated sheep, were assayed for testosterone and progesterone to confirm that the levels achieved were within the physiological range. Testosterone concentrations in plasma were measured using a "Coat-A-Count" solid-phase ^{125}I radioimmunoassay kit (Diagnostic Products Corporation, Calif., USA). The kit measured testosterone concentrations in unextracted plasma samples. All samples were analyzed in a single assay for which the reported intra-assay coefficient of variation was between 5 and 16%. The sensitivity of the assay was 0.44 ng/ml. Progesterone was measured in plasma samples using an extracted radioimmunoassay previously described by Rice et al. (1986). All samples were analyzed in a single assay for which the intra-assay coefficient of variability was determined to be 10.1%. The sensitivity of the assay was 0.15 ng/ml.

Statistics

All values are expressed as the mean ± standard error of the mean (SEM). Raw data were used for the analysis of sham and vehicle treatments in sheep with and without sex steroid replacement. To reduce within-subject variability, the vehicle, sertraline and imipramine treatment data were standardized prior to analysis of variance. Standardization involved calculating the mean of the four values obtained from plasma sampled during the 30 min preceding treatment and subtracting it from each of the post-treatment values measured for each animal. Analysis of variance was used to evaluate the effects of both imipramine and sertraline, with sex as the between-subjects factor, and sex steroid treatment, drug dose and time as within-subjects factors, and α set at 0.05 (Statistica v.5.5; Statsoft, Tulsa, Okla., USA). Post-hoc analyses were done using the least significant difference test, with α set at 0.05.

Results

Sex steroid replacement

The average testosterone level measured in castrated rams treated with testosterone implants was 6.37±0.45 ng/ml, which was significantly (P<0.05) higher than testosterone levels in the same rams in the absence of treatment with testosterone implants (less than 0.44 ng/ml). Testosterone levels for castrated rams treated with testosterone implants were within the physiological range of levels reported for intact rams (2–20 ng/ml; D'Occhio and Brooks 1982).

The average progesterone level measured in OVX ewes treated with exogenous progesterone implants was 3.65±0.6 ng/ml, which was significantly higher (P<0.05) than progesterone levels in the same ewes in the absence of treatment with progesterone implants (less than 0.15 ng/ml). Progesterone levels for ewes treated with progesterone implants were within the physiological range measured in normally cycling ewes during the luteal phase of the estrous cycle (3 ng/ml; Evans et al. 1994).

Actual (in intact sheep) and simulated (generated using implants in OVX sheep) luteal phase oestrogen levels are very low (1–2 pg/ml; Evans et al. 1994) and below the detection limit of our oestrogen assay.

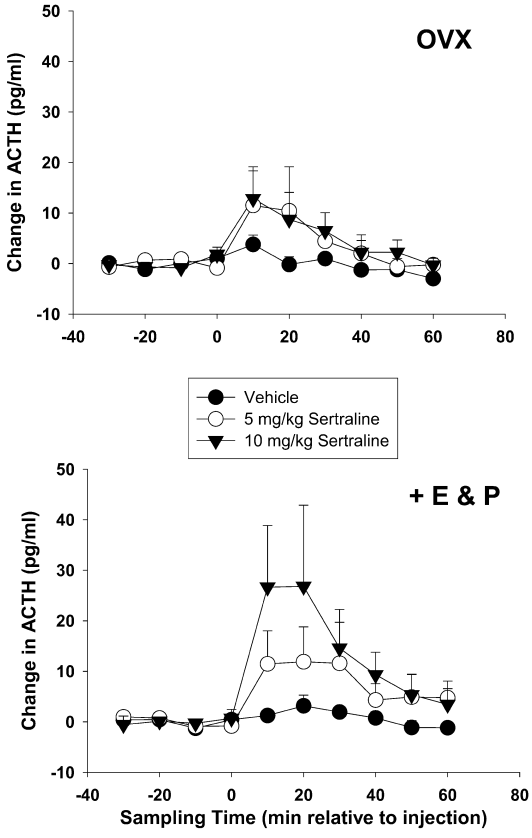

Fig. 1 ACTH responses to vehicle, 5 or 10 mg/kg sertraline relative to baseline following acute SC injection at time 0 in ovariectomized ewes with (*+E & P*) and without (*OVX*) sex steroid replacement (n=6). ACTH responses to vehicle were less than those to 5 mg/kg sertraline, which were, in turn, less than those to 10 mg/kg sertraline (P<0.05). There was no significant effect of sex steroid status on the responses to vehicle or sertraline

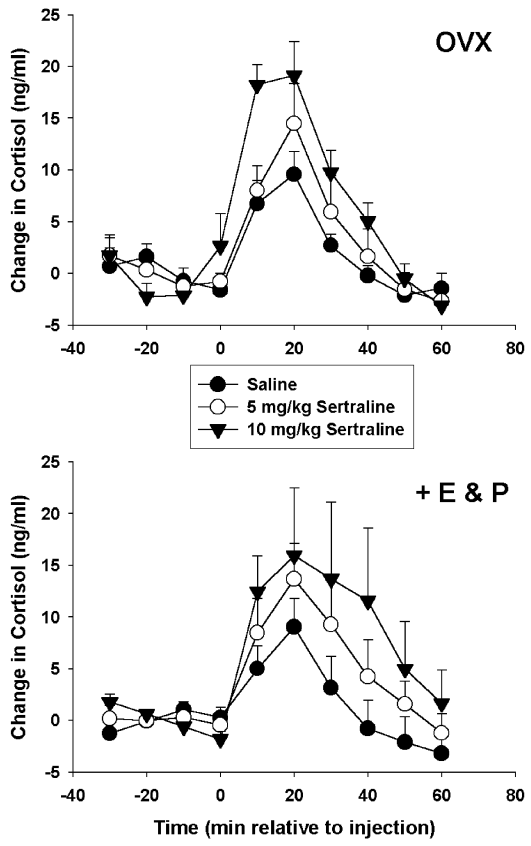

Fig. 2 Cortisol responses to vehicle, 5 or 10 mg/kg sertraline relative to baseline following acute SC injection at time 0 in ovariectomized ewes with (*+E & P*) and without (*OVX*) sex steroid replacement (n=6). Responses to vehicle were less than those to 5 mg/kg sertraline, which were, in turn, less than those to 10 mg/kg sertraline (P<0.05). There was no significant effect of sex steroid status on the responses to vehicle or sertraline

Behavioral observations

All sheep showed varying signs of discomfort after SC injection of the vehicle or drug. These signs were most pronounced immediately after injection and they included stamping of the feet, increased movement within the pen, occasional vocalization and signs of agitation and irritation that lasted for approximately 2–5 min. These behavioral changes appeared to be related to the SC injections, as there were no apparent differences in the behavioral responses amongst treatments.

Pretreatment ACTH and cortisol levels

The mean pretreatment ACTH and cortisol levels were 12.13±0.29 pg/ml and 7.58±0.30 ng/ml, respectively. There were no significant effects of sex, sex steroid replacement, drug treatment or time of sampling on baseline ACTH and cortisol levels.

Effect of sertraline treatment on ACTH and cortisol secretion

Standardized ACTH and cortisol results were compared for vehicle, 5 and 10 mg/kg sertraline treatments. There were significant [ACTH: $F(18,180)=2.89$, P<0.01; cortisol: $F(9,90)=2.04$, P<0.05] differences in the responses of castrated rams or OVX ewes with respect to the effects of sex steroid replacement and sertraline treatment over the sampling period.

Females. When given to OVX ewes, sertraline had a dose-dependent effect on ACTH secretion that was not affected by the presence or absence of oestrogen and progesterone replacement (Fig. 1). Both doses of sertraline (5 and 10 mg/kg) dose-dependently increased ACTH concentrations relative to when vehicle was administered [$F(18,90)=2.23$, P<0.01]. Sertraline treatment also resulted in a dose-dependent increase in cortisol concentrations, relative to that seen in response to vehicle treatment [$F(18,90)=2.63$, P<0.01]. Sex steroid treatment did not affect the cortisol response to sertraline in OVX ewes (Fig. 2).

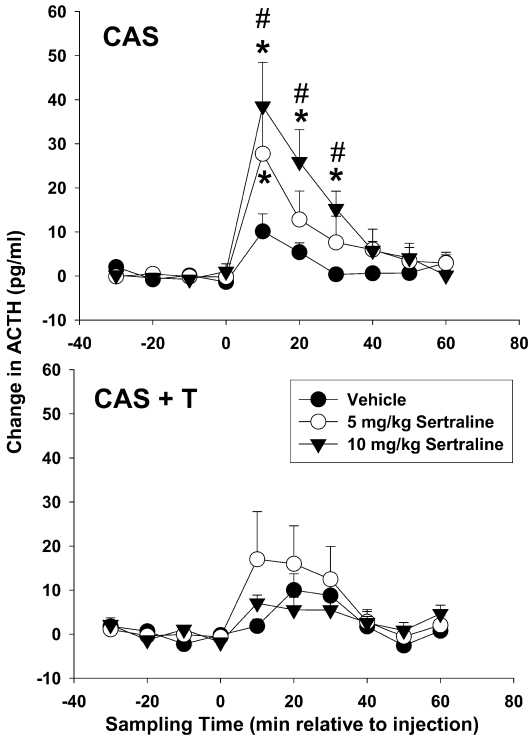

Fig. 3 ACTH responses in castrated rams (*CAS*) and castrated rams treated with testosterone (*CAS + T*) following a single SC injection of vehicle, 5 or 10 mg/kg sertraline at time 0 (n=6). Significantly different responses between the various treatments were seen in the absence, but not presence of T (*vehicle versus sertraline, # 5 versus 10 mg/kg sertraline:P<0.05)

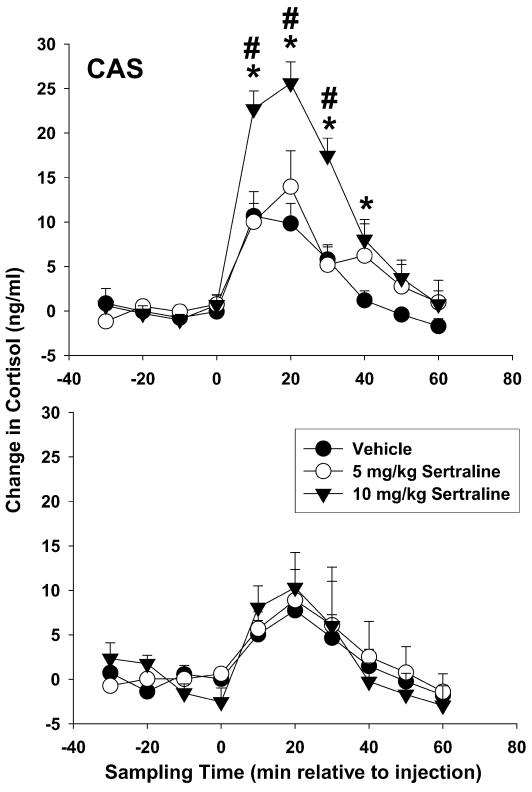

Fig. 4 Cortisol responses in castrated rams (*CAS*) and castrated rams treated with testosterone (*CAS + T*) following a single SC injection of vehicle, 5 or 10 mg/kg sertraline at time 0 (n=6). Significantly different responses between the various treatments were seen in the absence, but not presence of T (*vehicle versus sertraline, # 5 versus 10 mg/kg sertraline:P<0.05)

Males. In castrated rams, treatment with testosterone significantly affected the ACTH response to sertraline treatment. In the absence of testosterone, sertraline treatment was associated with a dose-dependent increase in ACTH concentrations [F(18,90)=4.03, P<0.01; Fig. 3a]. In contrast, when castrated rams were treated with testosterone, neither dose of sertraline increased ACTH concentrations above those seen in response to vehicle treatment alone (Fig. 3b). The effect of sertraline on cortisol secretion was also affected by exogenous testosterone treatment [F(1,5)=10.85, P<0.05]. In castrated rams not treated with testosterone, 10 mg/kg sertraline treatment led to significant increases in cortisol above those seen in response to vehicle or 5 mg/kg sertraline [F(2,10)=13.14, P<0.01; Fig. 4a]. When the rams were treated with testosterone, neither dose of sertraline increased cortisol concentrations (Fig. 4b).

Effect of imipramine treatment on ACTH and cortisol secretion

Standardized ACTH and cortisol results were compared for vehicle or 10 mg/kg imipramine. There was a significant effect of sex [F(1,10)=5.23, P<0.05], with rams having higher ACTH secretion overall. However, there was no evidence that imipramine treatment resulted in any change in ACTH secretion in gonadectomised rams or ewes, with or without sex steroid replacement (data not shown). In the case of cortisol, there was a significant effect of sampling time [F(9,90)=31.30, P<0.01], which indicated that cortisol release following vehicle or imipramine treatment (+10, +20 and +30 min) was greater than cortisol levels measured prior to treatment (−30 to 0 min). There were no significant interactions between sex, sex steroid replacement, treatment or sampling time (data not shown).

Discussion

This study examined the effects of sex and sex steroid replacement on the HPA axis response to acute treatment with the antidepressants sertraline and imipramine in the sheep. Our results support the hypothesis that sex is a determinant of the sensitivity of the HPA axis to stimulation by acute administration of sertraline in gonadectomised sheep. Ovariectomized (OVX) ewes were more sensitive to the HPA stimulatory effects of acute sertraline treatment than were castrated rams, as they responded with significant increases in ACTH and

cortisol to a lower sertraline dose (5 mg/kg) than did the rams. These results are consistent with an earlier study in which we reported that sertraline (2 and 5 mg/kg) stimulated ACTH and cortisol secretion in OVX ewes, but was ineffective in castrated rams (Broadbear et al. 2004). In the present study, a stimulatory effect of sertraline on ACTH and cortisol secretion was detected in males, but only when the dose of sertraline from our earlier study was doubled. However, the predictions regarding the effects of sex steroid replacement on the HPA responses to acute sertraline treatment were not supported by the results of this study. We anticipated that treatment of OVX ewes with mid-luteal levels of estradiol and progesterone would stimulate the ACTH and cortisol response following sertraline treatment. However, the dose-dependent stimulation of ACTH and cortisol release in response to sertraline was unaffected by simulated luteal phase sex steroid replacement in OVX ewes. In addition, although we predicted a neutral effect of testosterone replacement on any HPA response to antidepressant treatment, the results of this study clearly show that testosterone replacement abolished the stimulatory HPA axis response to sertraline administration that was measured in the absence of testosterone replacement in castrated rams. These results have important implications for our understanding of the role of sex steroids in regulating neuroendocrine responses of the HPA axis, and may go some way toward explaining the differences in the efficacy of antidepressant treatments between men and women that are sometimes reported.

The treatment of OVX ewes with sex steroids in the present study did not change the ACTH and cortisol responses to sertraline. The levels of oestrogen and progesterone used in this study were chosen to mimic the luteal phase of the reproductive cycle (Evans et al. 1994). While the within-subject design of this study is one of its strengths as it maximized our ability to detect treatment differences, a between-subject design using intact sheep may have provided additional information, although previous comparisons of sex steroid-treated and intact sheep have yielded essentially the same results (see below). Another limitation of this study was that we did not evaluate the effects of follicular phase levels of oestradiol, nor of oestradiol or progesterone treatments given alone, on HPA axis responsiveness to sertraline treatment. A number of reports have addressed the separate contributions of oestradiol and progesterone to the regulation of the HPA axis in sheep. For instance, when Komesaroff et al. (1998) treated OVX ewes with follicular phase levels of oestradiol in the absence of progesterone, the glucocorticoid response to either audiovisual stress or insulin-induced hypoglycaemia was attenuated. This was consistent with outcomes using intact ewes that were tested across the oestrous cycle, in which ACTH and cortisol responses to both stressors were greater during the luteal phase than in the follicular phase (Komesaroff et al. 1998). A study by Keller-Wood (2000) found that whilst oestradiol treatment did not change ACTH secretion in response to hypotension in OVX ewes, the duration of the ACTH response to hypotension in progesterone-treated OVX ewes was greater than in intact ewes. The findings of these studies imply that if sex steroids do enhance the HPA axis sensitivity to serotonergic input, this should have been observable under the luteal phase conditions simulated in the present study. Tilbrook et al. (1999a) investigated the contribution of sex and sex steroid replacement to cortisol responses during isolation/restraint stress. Their study included OVX ewes treated with oestradiol, progesterone or oestradiol and progesterone. No differences were found in the cortisol responses to isolation/restraint stress for the sheep in the different sex steroid replacement groups (Tilbrook et al. 1999a), nor between intact and simulated luteal phase ewes (Turner et al. 2002). These findings in sheep are in contrast to rat studies, in which it has been reported that oestrogen increases the activity of the HPA axis and the glucocorticoid response to stress (Viau and Meaney 1991; Bingaman et al. 1994), while progesterone decreases glucocorticoid secretion (Redei et al. 1994).

Sertraline treatment had dramatically different effects on ACTH and cortisol secretion in castrated rams in the presence and absence of exogenous testosterone. In the absence of testosterone, castrated rams responded with significant increases in ACTH and cortisol following treatment with the highest dose of sertraline. In the presence of testosterone, however, castrated rams were unresponsive to the stimulatory HPA effects of sertraline. Therefore, testosterone appears to have an inhibitory role in the 5-HT pathway through which sertraline-enhanced 5-HT levels activate the HPA axis. Tilbrook et al. (1999a) investigated the contribution of sex and sex steroid replacement in gonadectomised sheep to cortisol responses during isolation/restraint stress. Their study included castrated rams treated with testosterone (twice-daily injections of 6 mg testosterone propionate). No differences were found in the cortisol responses to isolation/restraint stress for the sheep in the presence or absence of testosterone (Tilbrook et al. 1999a). This was true also for cortisol responses in castrated rams following intracerebroventricular administration of CRH and/or AVP, for which no effect of testosterone replacement was noted (Tilbrook et al. 1999b). In addition, Turner et al. (2002) reported no difference in cortisol response to isolation/restraint stress or insulin-induced hypoglycaemia in intact and gonadectomised rams. Therefore, our finding is somewhat novel in the context of earlier work done in sheep. Our use of testosterone pellets may have generated higher plasma levels of testosterone than the twice-daily injections of testosterone propionate (TP) used in the earlier sheep studies. For example, the doses of TP used by the Tilbrook group (1991, 1999a,b) generated plasma testosterone levels of less than 2 ng/ml as opposed to 6.4 ng/ml in the present study. The use of sertraline as an HPA axis stimulus may also differ from isolation/restraint or other physical or psychological stressors with respect to the mechanistic pathway through which increased ACTH and cortisol secretion was triggered. Our results are actually more consistent with data obtained in the rat, since castration of

adult male rats enhances the ACTH and corticosterone responses to acute stress (Handa et al. 1994) and increases both hypothalamic CRH levels and CRH immunoreactivity (Bingaman et al. 1994). These changes following castration can be blocked by the administration of 5α-dihydrotestosterone, the non-aromatizable form of testosterone, which implicates androgen rather than oestrogen receptor involvement at or above the hypothalamic level of the HPA axis. More recently, Viau and Meaney (1996) demonstrated that testosterone-related changes in the magnitude of the ACTH response to stress are positively correlated with AVP rather than CRH stores in the median eminence of the paraventricular nucleus (PVN) of the hypothalamus. They concluded that the changes in AVP are due to testosterone-mediated changes in glucocorticoid feedback in the medial preoptic area (MPOA; Viau and Meaney 1996). Androgen and oestrogen receptors have both been identified in the ram MPOA (Scott et al. 2000). Therefore, it can be speculated that testosterone is acting via a similar mechanism in both sheep and rat to attenuate the HPA axis response in sertraline-treated sheep.

Despite this difference in HPA axis activation between male and female sheep, acute treatment with sertraline resulted in a similar increase in prolactin secretion in all sheep (Broadbear et al. 2004). The prolactin results imply that there was no sex difference in the effects of sertraline on 5-HT availability per se, but that the sex difference measured in the HPA axis response to sertraline probably occurred at the pituitary or hypothalamic level. Serotonin is released from serotonergic nerve terminals in the paraventricular nucleus of the hypothalamus (PVN), where it interacts with CRH containing neurons. 5-HT stimulates CRH neuron firing, producing an increase in CRH release that then activates the HPA axis (Holsboer and Barden 1996). SSRIs such as sertraline, which elevate 5-HT levels by blocking reuptake of 5-HT from the synaptic cleft, are known to stimulate ACTH and cortisol secretion (Meltzer et al. 1987; O'Keane et al. 1991).

In this study, the effect of imipramine treatment on cortisol secretion was no different from vehicle treatment regardless of sex or sex steroid status. In our earlier study, in which the effects of lower doses of sertraline and imipramine on ACTH, cortisol and prolactin were measured, treatment with imipramine did not change any of the neuroendocrine markers (Broadbear et al. 2004). Imipramine does not always stimulate the HPA axis when it is administered via non-intravenous routes (Dredge et al. 1999), and our conclusion was that the dose used in this earlier study was inadequate. As use of the intravenous route for administration of imipramine is contraindicated in sheep (Meineke et al. 1997), we doubled the dose of SC imipramine used in the present study. Despite the fact that this dose (10 mg/kg) was sufficient to produce cardiotoxicity in sheep via the intravenous route (Meineke et al. 1997), we saw no HPA axis activation following imipramine administration via the SC route. Since it has been shown that intracerebroventricular administration of both epinephrine and norepinephrine leads to HPA axis activation in sheep (Lui et al. 1991), we conclude that imipramine is either inactive in the sheep, or poorly absorbed via the SC route.

While the majority of sex-related differences in physiology can be attributed to the different sex steroids produced by the ovaries and testes, it is important to recognize that other gonadal factors may be involved. Sex steroid replacement regimens are, at best, mimics of physiological processes, as it is virtually impossible to faithfully reproduce the normal, pulsatile changes in hormone concentrations seen in vivo. In addition, a number of sex differences are mediated or imprinted during early stages of physical and neuronal development and may not be erased by removal of the gonads (Canny et al. 1999). This is also evident from the sex difference observed in our earlier study using gonadectomised sheep (Broadbear et al. 2004). Therefore, in order to gain a complete understanding of how an individual's sex and sex steroids determine their physiology, additional studies using intact sheep are needed to determine whether the conclusions derived from the present study can still be applied when testing takes place under normal physiological conditions.

In conclusion, the current study presents a novel approach to the identification of sex differences following acute administration of sertraline and imipramine on HPA axis activity with and without sex steroid replacement in gonadectomised sheep. The HPA axes of male and female sheep were activated in response to sertraline treatment, most likely as a result of an increase in serotonin levels that stimulated the hypothalamic release of CRH. Furthermore, the HPA effects of sertraline treatment differed between males and females. In OVX ewes, the dose-dependent increase in HPA axis activity following sertraline was not affected by treatment with simulated luteal phase levels of oestrogen and progesterone. In castrated rams however, testosterone replacement abolished the stimulatory effects of sertraline treatment on ACTH and cortisol that were present in the absence of testosterone replacement. While it is unclear whether the sex differences in disease incidence and treatment outcomes is a simple reflection of differences in the physiology of males and females, or is a consequence of complex social and biological determinants, the clear relationship between hormonal status in women and likelihood of depression, as well as the evidence for sex differences in antidepressant efficacy, underscores the importance of the basic research that is needed to reveal the mechanisms through which serotonin, stress and sex hormones interact.

Acknowledgments The authors wish to thank Isabella Retamal for her excellent technical assistance. Many thanks to Bruce Doughton and the staff at the Prince Henry's Institute of Medical Research facility at Werribee for assistance with the sheep. We gratefully acknowledge the provision of sertraline hydrochloride by Pfizer Pty Ltd, West Ryde, Australia.

Animals used in these studies were maintained in accordance with the "Principles of laboratory animal care" (NIH publication No. 83-23, revised 1985), and with prior institutional animal ethics committee approval in accordance with National Health and Medical Research Council of Australia guidelines.

Financial support for this work was provided by the National Health and Medical Research Council of Australia, Grant #143547.

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