radicals. The involvement of cytochrome *b* in the active oxygen-generating system has also been proposed[9-11], but it is not known whether cytochrome *b* activates oxygen molecules by forming oxygenated cytochrome *b*, as in cytochrome oxidase[21,22]. If the redox midpotential of the cytochrome *b* were nearly as high as that of cytochrome oxidase or the steric factor of the cytochrome *b* permitted, BW755C and PPDA would interact with the cytochrome *b*. There is another possibility for their pharmacological actions; these drugs could be activated by mitochondrial cytochrome oxidase to act as radical scavengers. This may be supported by the short-termed appearance of a compound possessing an absorption maximum at 524 nm. For the better understanding of the molecular mechanism of the potent inhibition of BW755C on the cyclo- and lipo-oxygenases in macrophages, more extensive studies with macrophages are needed; such studies are currently being carried out in our laboratory.

Abbreviations. PPDA, p-phenylenediamine; NSAIDS, non-steroidal anti-inflammatory drugs; MEM, minimum essential medium.

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Androstenedione or corticosterone treatment during pregnancy alters estrous cycle of adult female offspring in mice

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Summary. Female offspring from mice injected with androstenedione during late pregnancy showed lengthened vaginal cycles, persistent estrus and decreased incidence of pro-estrus and diestrus, whilst offspring from mice injected with corticosterone showed increased incidence of diestrus. These observations give qualified support to the hypothesis that stress during pregnancy alters the female offspring reproductive system through the action of adrenal steroids.
Key words. Mouse, female offspring; androstenedione treatment; corticosterone treatment; pregnancy; estrous cycle.

The effects of stress during pregnancy in reducing sexual behavior of the male offspring in rodents is well known[1-3]. Ward[1] and Dahlöf et al.[2] have suggested that this effect is mediated by increased maternal pituitary-adrenocortical output and that in utero exposure to adrenal steroids compromises sexual-behavioral differentiation of the fetal male: we have recently contributed evidence supporting this hypothesis[3]. Similarly, evidence exists to suggest that stress during pregnancy influences sexual development of female offspring: in rats, prenatal stress lengthens the estrus-metestrus stages of the adult cycle[4], whilst in mice prenatal stress shortens the length of the pro-estrus stage and delays puberty[5]. No experiments to date have tested the hypothesis that the effects of stress during pregnancy upon female offspring reproductive function are mediated by in utero exposure to maternal adrenocortical products. In rats and mice the major adrenocortical product released in the stress response is corticosterone[6], but androgens, estrogens and progestagens can also be secreted from the adrenal cortex under the influence of ACTH[7,8]. Stress during pregnancy increases maternal and fetal plasma androstenedione concentrations in the rat[9], and it has been suggested that fetal exposure to this compound disrupts sexual differentiation[1,9]. Additionally, as corticosterone[10] and most other unbound steroids cross the placenta[11], it is possible that fetal exposure to other adrenocortical products may mediate the effects of stress during pregnancy upon the female offspring. The purpose of this study was to examine the effects of androstenedione or corticosterone administration during late pregnancy, upon the estrous cycle of adult female offspring.

Materials and methods. Females used were virgin TO mice (A. Tuck and Sons, Battlesbridge, Essex), obtained two weeks prior to mating. They were housed in groups of 10 in large plastic cages (42 × 25 × 11 cm), allowed ad libitum supply of food (Labsure animal diet, Christopher Hill Ltd, Dorset) and water, and maintained on a reverse lighting regime (red lights on 12.00–22.00 h) at 18–23 °C. At 10–12 weeks of age, these females were placed individually into small plastic cages (30 × 13 × 11 cm) with a male and observed daily for the appearance of a vaginal plug, which was deemed to indicate day 0 of pregnancy. Males were then removed and females were left undisturbed until day 12 of pregnancy when treatments were administered. Pregnant females were randomly assigned to 1 of 4 treatments: untreated controls (n = 8), steroid vehicle injection controls (n = 7), corticosterone treatment (n = 8) and

androstenedione treatment (n = 7). Each female in the corticosterone group received injections of 100 µg corticosterone (Sigma) in 0.1 ml vehicle; androstenedione treated females received injections of 100 µg androstenedione (Sigma) in 0.1 ml vehicle, and the vehicle group received 0.1 ml vehicle only. The vehicle was peanut oil (Sigma) and injections were given s.c., daily between 15.00 and 16.00 h, on days 12–17 of pregnancy. Untreated control females were left undisturbed throughout this period. At birth, litters were randomly culled to eight pups and fostered to an untreated female that had given birth within the previous 24 h. This procedure controls certain postnatal influences upon offspring development, such as continued exposure to steroids in milk, and poor lactation or maternal care due to steroid treatment. Litters were weaned on postnatal day 21 and female offspring were rehoused in small groups according to treatment. When approximately eight weeks old, female offspring were rehoused individually in small cages for eight days prior to study. Daily smears were taken from these animals between 10.00 and 12.00 h by vaginal lavage for 21 days. Dry smears were stained with Giemsa (BDH, 1:20 in water) and staged according to Bingel and Schwartz[12]. Bedding was changed regularly (7–10-day intervals) and care was taken throughout all experiments to avoid contamination or contact with male bedding, which may influence estrous cycles[13,14]. Statistical analysis of results employed one way analysis of variance (IWANOVA) and t-tests; proportional differences were analyzed using Fisher's Exact Probability test.

Results. The effects of the maternal androstenedione and corticosterone administration upon the estrous cycles of offspring are shown in the table. Androstenedione-exposed females showed decreased incidence of pro-estrus and diestrus smears and increased incidence of estrus smears, whilst corticosterone-exposed females showed increased incidence of diestrus smears. Almost half the androstenedione-treated females (42.8%) showed persistent estrus lasting for six or more days, but this condition was not seen at all in the control, oil and corticosterone groups ($p = 0.008$). Additionally, a large proportion (92.8%) of the androstenedione offspring showed evidence of cycles lacking a clear pro-estrus stage: this condition was uncommon in other groups (control 21.1%; vehicle control 14.2%; corticosterone 35.7% ($p = 0.0001$)). This result does not represent absence but rather shortening of this cycle stage so that its presence is undetected during the daily smearing regime. Androstenedione females also had longer cycles and substantially more of these animals (35.7%) had cycles so lengthened that a complete cycle could not be observed during the 21-day smearing period (see table).

Number of days of 21-day test period in each cycle stage

Treatment	Pro-estrus	Estrus	Metestrus	Diestrus	Cycle length (days)
Control n = 14	3.93 ± 0.29	5.57 ± 0.36	6.43 ± 0.27	5.07 ± 0.29	5.15 ± 0.17
Vehicle n = 14	3.64 ± 0.25	5.36 ± 0.36	6.07 ± 0.49	5.85 ± 0.42	6.14 ± 0.85
Androstenedione n = 14	1.78** ± 0.50	11.57*** ± 1.06	5.14 ± 0.58	2.50*** ± 0.53	6.46[a] ± 0.63
Corticosterone n = 14	3.71 ± 0.19	5.07 ± 0.35	5.71 ± 0.29	6.42* ± 0.44	4.95 ± 0.10
IWANOVA	$p < 0.0001$	$p < 0.0001$	NS	$p < 0.0001$	NS

The effects of androstenedione and corticosterone administration during late pregnancy upon the estrous cycle of individually housed offspring. Data presented are as means ± SEM.
* Significant difference compared with control $p < 0.01$ (t-test).
** Significant difference compared with control $p < 0.001$ (t-test).
*** Significant difference compared with control $p < 0.0005$ (t-test).
[a] 5 females failed to show a complete cycle $p = 0.02$ (Fishers test).
Mean cycle lengths were calculated omitting data from these animals. NS, not significant.

Discussion. This study gives evidence that fetal exposure to adrenocortical steroids alters later reproductive function in females, and as such supports the hypothesis that the effects of stress during pregnancy may be mediated by these compounds. Androstenedione-exposed females showed disruptions of the estrous cycle most closely resembling the effects of stress: decreased incidence of pro-estrus stages[5] and increased duration of estrus stages[4]. However, the length of the metestrus stage and of the entire cycle (in those animals completing cycles during the test period), were not influenced.

In both rats and mice gonadotrophin regulating structures are functional before birth: LH, FSH and PRL are released from the fetal pituitary[15–17]. Additionally, estrogen receptors are present in the fetal hypothalamus[18], and development of the hypothalamic-pituitary systems regulating reproductive function is completed during the early neonatal period[19]. There is thus the potential for disruption of these developmental processes by exposure to abnormal concentrations of circulating steroids during intra-uterine life.

It has previously been shown that moderate exposure of the female fetus in utero to testosterone from adjacent males lengthens the estrous cycle[20], and exposure of the male fetus to androstenedione masculinises later behavioral responses[21]. Presumably, these effects are mediated by the aromatized estrogenic compounds of androgens[22]. Exposure of the female fetus to elevated concentrations of estrone (derived from androstenedione)[22], at a time when estrogen receptors are present in the hypothalamus and when gonodotrophin-regulating structures are developing, may well impair later reproductive function by altering feedback control of the hypothalamic-pituitary-ovarian axis.

Prenatal corticosterone exposure probably influences female reproductive function via a different mechanism. Glucocorticoid exposure, perinatally, delays brain development[23], and results in disturbances in brain nucleic acid ratios, protein and tubulin content[24–26]. Therefore the effect of corticosterone upon the developing brain may be to impair development and function of maturing neuroendrocine systems, and desynchronize the processes of sexual differentiation. A second peripheral effect of corticosterone exposure early in life, is to prevent proper development of the adrenal[27]. Considering the importance of the adrenal in reproductive development[28] and the cyclic changes in corticosterone throughout the mouse estrous cycle[29], impaired adrenocortical output may result in alterations of endocrine control of the estrous cycle.

The treatments described above produce effects in females that in some ways resemble those of stress during pregnancy. However, the full details of the endocrine causation, and indeed the endocrine consequences, of stress before birth, remain to be discovered.

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Hyperprolactinemia and estrogen-induced rhythms in LH and prolactin release in the ovariectomized rat

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Summary. Short-term (9 days) hyperprolactinemia induced by pituitary grafts reduced basal plasma LH levels in ovariectomized rats whereas long-term (31 days) grafts increased basal LH levels. Although long-term grafts inhibited estradiol-induced prolactin surges, hyperprolactinemia had no effect on the LH surge. It is concluded that the estrogen-treated ovariectomized rat is not suitable for studying the effects of hyperprolactinemia on LH release.
Key words. Hyperprolactinemia, LH and prolactin.

Interference with ovulatory cycles is a frequent clinical manifestation of hyperprolactinemia in women. The underlying mechanisms responsible for this disruption remain unclear. Both exaggerated[2] and impaired[3] release of LH in response to luteinizing hormone releasing hormone have been reported.

Various animal models have been utilized to investigate the possible mechanisms involved. In intact female rats, hyperprolactinemia produced by prolactin-secreting tumors[4,5] pituitary grafts or dopamine antagonists[6] has been shown to suppress basal plasma levels of LH and to cause acyclicity. However, it has been questioned whether the intact female rat is a suitable model for studying the suppression of LH release by elevated plasma prolactin levels since this may lead to pseudopregnancy associated with increased progesterone secretion[7]. In the chronically ovariectomized rat, prolonged hyperprolactinemia does not affect basal plasma LH levels[8,9]. Recent studies indicate however, that steroid-induced release of LH may be selectively reduced by hyperprolactinemia[9], suggesting that this may be a more relevant model. This idea is further supported by findings that estrogen-induced positive feedback of LH release is inhibited in hyperprolactinemic patients[10]. On the other hand, some recent reports argue against the validity of this model[11,12].

The major aim of the present study was to determine whether hyperprolactinemia induced by anterior pituitary grafts could suppress the circadian rhythm of LH or prolactin release following administration of estradiol to ovariectomized rats.

Materials and methods. Animals. Female Wistar AG rats were obtained from C.S.E.A.L.-C.N.R.S. (Orleans) at 10–12 weeks of age. Following bilateral ovariectomy, the animals were kept under standardized lighting conditions (lights on 05.00–17.00 h) and provided with food and water ad libitum.

Surgical procedures. Three to four weeks after ovariectomy, the animals were assigned to one of three groups which were treated as follows: Group 1 (control): rats (n = 22) received a sham graft (fat tissue) under kidney capsule on day 0 of the experiment. A Silastic tube (Dow Corning 601-321, 0.5 cm in length) filled with estradiol was implanted s.c. in the back[13] 4 or 26 days later. Three days after the implant, an indwelling cannula was placed in a jugular vein. All of the above procedures were carried out under ether anesthesia. Two days after cannulation, blood samples of 0.3–0.4 ml were taken via the cannula at 2-h intervals beween 09.00 and 17.00 h. The samples were centrifuged and the plasma stored at −20 °C until assayed for prolactin and LH concentrations.

Group 2 (short-term graft): rats (n = 8) received a transplant consisting of three anterior pituitaries from donor rats under the kidney capsule on day 0 of the experiment. An estradiol implant was made four days later as described above. The animals were then treated exactly as the rats in group 1.

Group 3 (long-term graft): rats (n = 16) received a transplant of three anterior pituitaries, and estradiol was implanted 26 days later. The animals were then treated exactly as the rats in group 1.

Chemical assays. Plasma levels of LH and prolactin were determined by radioimmunoassay according to the methods of Niswender et al.[14,15]. All results were expressed in terms of NIAMDD rat LH-RP-1 and NIAMDD rat PRL-RP-1, respectively.

Statistical analysis. The data were analyzed initially by 2-way analysis of variance (treatment, time of day) with subsequent analysis of each variable by 1-way analysis of variance and Student's t-test.

Results. The plasma levels of prolactin throughout day 5 after estradiol implantation for the various treatment groups are shown in figure 1. All of the experimental groups which had received pituitary grafts had significantly elevated basal levels of prolactin at 09.00 h when compared with the sham control group. Preliminary studies (not shown) indicated that implantation of estradiol had no effect on the graft-induced elevation

Effect of anterior pituitary grafts on basal plasma levels of LH in ovariectomized rats with estradiol implant

Duration of graft	Plasma LH
0 (sham)	161 ± 16[a] (22)
9 days	105 ± 8[b] (8)
31 days	264 ± 21[b] (16)

Anterior pituitary or sham grafts were made as described in Methods. Five days after implantation of estradiol, blood was collected at 09.00 h. Numbers in parentheses represent the number of animals. [a] ng/ml + 1 SE. [b] Significantly different from control ($p < 0.05$).