Hormonal changes in the immature rat after administration of pregnant mare serum gonadotrophin: influence of body weight

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SUMMARY

We have shown previously that pregnant mare serum gonadotrophin (PMSG) induces ovulation only in rats weighing over 60 g on the day of injection. The under-60 g rats do not ovulate although they secrete a preovulatory surge of a pleiomorphic form of LH. Presumably this pleiomorph is inactive.

Comparisons were made of plasma hormone concentrations in rats treated with PMSG that weighed over and under 60 g. The measurements were made on samples taken between 13.00 and 22.00 h on the day of the expected preovulatory LH surge. Prolactin and corticosterone levels were lower in the lighter group compared with the heavier group. A midday pulse of GH detected in the over-60 g animals did not occur in the under-60 g group. Levels of ACTH were slightly higher in the under-60 g rats and together with the low corticosterone concentrations indicate adrenal insensitivity. Oestradiol, progesterone and TSH concentrations were the same in the two groups. Since progesterone secretion is under LH control, the ‘inactive’ pleiomorphic form of LH must have steroidogenic activity. There was an indication that the under-60 g rats also secreted a pleiomorphic form of FSH.

Reports in the literature indicate that prolactin, corticosterone and GH have a positive modulatory influence on natural puberty. They may also influence precocious puberty induced by PMSG, since in the unresponsive under-60 g rat plasma levels of these three hormones were low. Perhaps the release of one or more of these hormones is dependent upon the physical maturity of the animal as represented by body weight.

INTRODUCTION

Administration of a single injection of pregnant mare serum gonadotrophin (PMSG) to female rats from approximately day 20 of life can induce an increase in secretion of gonadotrophins and steroids leading to ovulation (Zarrow & Quinn, 1963; Parker, Costoff, Muldoon & Mahesh, 1976). The pattern of hormone secretion is similar to that in the oestrous cycle of the adult rat and before the first ovulation at puberty (Butcher, Collins & Fugo, 1974; Meijis-Roelofs, Uilenbroek, de Greef, de Jong & Kramer, 1975; Parker & Mahesh, 1976). Thus the PMSG-treated immature rat has been used as a model for studying the control of the onset of puberty (see Ramaley, 1974).

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Injection of PMSG causes, within 20–24 h, a marked rise in plasma oestrogen concentrations which reach a maximum at approximately 48 h (Wilson, Horth, Endersby & McDonald, 1974; Shasida & Johnson, 1976). This is followed by an increase in hypothalamic gonadotrophin releasing hormone concentration (Sorrentino & Sundberg, 1975) and stimulation of the release of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin from the pituitary gland (Johnson, Reiter & Blask, 1975; Sasamoto & Johke, 1975), and various gonadal and adrenal steroids, including progesterone and testosterone, at 52–56 h after PMSG (Wilson et al. 1974; Parker et al. 1976). Ovulation occurs 12 h after the release of gonadotrophin.

In previous reports we have shown that administration of 5–20 i.u. PMSG induces ovulation on day 27 of life in Sprague–Dawley rats, and on day 30 in Wistar rats, as long as they weigh 60 g or more on the day of injection. Those weighing less than 60 g do not ovulate and follicular rupture does not take place (Wilson, 1970; Wilson & Endersby, 1979), despite an apparent LH surge occurring 52–54 h after the PMSG, as measured by heterologous radioimmunoassay. Using an homologous radioimmunoassay no LH surge was detected and this suggests that a pleiomorphic form of LH is released (ter Haar & Wilson, 1978).

We have studied the underweight immature rats further to ascertain whether their failure to ovulate in response to PMSG is due to one of the following: (1) a lack of maturation of some physical aspect of the animal which is seen finally as a low body weight or (2) a lack of a circulating hormone which is necessary to sensitize the ovary towards the endogenous gonadotrophins or to stimulate the pituitary gland to release an active pleiomorphic form of LH. In the first experiments the percentage body composition of the under-60 g rat was exactly the same as the over-60 g rat, but the under-60 g rat had a significantly lower body core temperature and oxygen consumption. We found that raising the former but not the latter induced ovulation in the under-60 g PMSG-treated rat (Everard, Rothwell, Stock & Wilson, 1983). The second possibility was investigated in two ways, firstly by injecting a variety of hormones on the day of the expected LH surge in under-60 g PMSG-treated rats to see whether ovulation could be induced. Several hormones were effective, including gonadotrophins, prolactin, growth hormone (GH), tri-iodothyronine, corticosterone and any hormone that stimulated the secretion of the latter (C. A. Wilson, unpublished results). The ovaries were thus capable of sustaining ovulation if given a sufficient and/or correct stimulus. The second approach was to compare circulating plasma levels of endogenous hormones in the under- and over-60 g rats after treatment with PMSG. We now report the influence of body weight on the secretion of various hormones in response to PMSG and discuss the possible relevance of this to the final ovulatory response.

MATERIALS AND METHODS

Sprague-Dawley rats (Tuck & Son, Rayleigh, Essex) were bought at 22 days of age and maintained in a lighting system of 14 h light: 10 h darkness (lights on 06.00–20.00 h). On day 27 of life they were weighed and injected subcutaneously at 12.00 h with either 0·1 ml 0·9% (w/v) NaCl or 5 i.u. PMSG (Folligon; Intervet Labs Ltd, Cambridge) in 0·1 ml saline.

On day 29, groups of animals were decapitated at 1-h intervals and the blood was collected from the severed neck, centrifuged at 400 g at 4 °C for 15 min and the plasma stored at −20 °C until assayed. The groups consisted of over- or under-60 g rats treated with PMSG or over-60 g rats treated with saline. The range of times of autopsy depended on the hormone to be assayed in the plasma. For instance, oestradiol levels rise in the morning and fall by late afternoon on the day of the expected LH surge (day 29) and so plasma samples were collected between 10.00 and 17.00 h. On the other hand, many of the pituitary hormones and progesterone rise in the late afternoon and evening hours of day 29 and so samples were collected between 13.00 and 21.00 h (see Introduction for references).
Corticosterone alters its circadian pattern of secretion after PMSG (Ramaley & Bartosik, 1975a) and so samples for measuring adrenocorticotrophin (ACTH) and corticosterone were taken over the morning, afternoon and evening hours (10.00–21.00 h).

**Hormone assays**

Plasma concentrations of FSH, thyroid-stimulating hormone (TSH), prolactin and GH were all measured by radioimmunoassay using reagents and protocols supplied by the NIADDK (Bethesda, Maryland, U.S.A.) and Dr A. F. Parlow (UCLA, U.S.A.). The details of the prolactin and TSH assays and the FSH assay using antiserum FSH-S₆ have been reported previously (Brown-Grant & ter Haar, 1977). The NIADDK radioimmunoassay for plasma FSH using antiserum FSH-S₁ had intra- and interassay coefficients of variation of 11 and 16%, respectively and a sensitivity of 65 µg/l. The GH measurements were made using rat GH-RP-1 as the reference preparation and anti-rat GH-S₄ as the antiserum preparation. Intra- and interassay coefficients of variation were 9.5 and 15.6%, respectively, and the sensitivity of the assay was approximately 150 ng/l. The plasma oestradiol concentration was also measured by radioimmunoassay, according to the method described by Bonney, Dixon & Fleming (1980) except that 0.2 ml plasma and 2.0 ml ether were used instead of 0.1 ml and 1.0 ml respectively. The procedure for the radioimmunoassay of progesterone was based on the World Health Organization system for matched reagents and has been described by Hodges, Eastman & Jenkins (1983). The intra- and interassay coefficients of variation were 8.7 and 9.6%, respectively.

Adrenocorticotrophin was measured by a cytochemical bioassay described by Chayen, Loveridge & Daly (1972) and Alaghband-Zadeh. Daly, Bitensky & Chayen (1974) and corticosterone by a fluorescent method (Zenker & Bernstein, 1958). Prolactin, FSH and TSH were all measured on the same samples, ACTH and corticosterone were measured on another set, while oestrogen, progesterone and GH were each measured on separate sets of samples.

**Statistical methods of analysis**

Comparisons of plasma concentrations between groups were made by Scheffé’s test after two-way analysis of variance adapted for unequal groups. Comparison of concentrations between times of day within a group were made by Student’s t-test. Table 1 shows the significance of the F values when two-way analysis of variance was carried out on the plasma hormone concentrations throughout day 29 in the three groups of rats. The method and relevance of this test is given in Snedecor & Cochran (1967).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Between groups (d.f. = 2)</th>
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<th>Interaction of group and time (d.f. = 16 or 18)</th>
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<td>GH</td>
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<td>TSH</td>
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<td>P&lt;0.01</td>
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<td>Progesterone</td>
<td>P&lt;0.01</td>
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NS, Not significant.
**RESULTS**

*Hormonal changes on day 29 after injection of 5 i.u. PMSG on day 27 to over- and under-60 g rats*

*Follicle-stimulating hormone (Fig. 1)*

Using the NIADDK assay with the S₆ antibody, FSH levels were stable at around 200 µg/l between 13.00 and 21.00 h on day 29 in untreated and under-60 g PMSG-treated rats. In the over-60 g PMSG-treated rats levels were similar in the early hours of the afternoon, but then a surge occurred with a rise starting at 18.00 h and a peak at 19.00–20.00 h. On another set of samples using another assay with S₁ antibody, FSH concentrations in the under-60 g rats only were measured. Again, levels were around 200 µg/l but a small rise ($P<0.05$) occurred at 20.00 h ($342±51$ µg/l) compared with levels at 17.00 h ($207±19$ µg/l).

Fig. 1. Changes in plasma FSH on day 29 of life in rats weighing under or over 60 g treated with 5 i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Over-60 g PMSG-treated rats (4–12 samples per time-point) using S₆ antiserum (○); under-60 g PMSG-treated rats (4–10 samples per time-point) using S₆ antiserum (●); untreated rats (4–10 samples per time-point) using S₆ antiserum (□); under-60 g PMSG-treated rats (4–6 samples per time-point) using S₁ antiserum (■). The vertical lines indicate ± s.e.m. *$P<0.05$, **$P<0.01$ compared with untreated group at same time (Scheffé’s test). †$P<0.05$, ‡$P<0.005$ compared with under-60 g PMSG-treated group at same time (Scheffé’s test). ††$P<0.05$ compared with same group at 17.00 h (Student’s $t$-test).
Hormonal changes after PMSG treatment

Fig. 2. Changes in plasma prolactin on day 29 of life in rats weighing under or over 60 g treated with 5i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Untreated rats (4–10 samples per time-point) (□); over-60 g PMSG-treated rats (4–11 samples per time-point) (○); under-60 g PMSG-treated rats (4–10 samples per time-point) (●). The vertical lines indicate ± S.E.M. * P<0.05, ** P<0.01, *** P<0.005, **** P<0.001 compared with untreated group at same time (Scheffé's test). † P<0.05, ‡‡ P<0.01, ‡‡‡ P<0.005 compared with under-60 g PMSG-treated group at same time (Scheffé's test).

Fig. 3. Changes in plasma TSH on day 29 of life in rats weighing under or over 60 g treated with 5i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Untreated rats (4–10 samples per time-point) (□); over-60 g PMSG-treated rats (4–12 samples per time-point) (○); under-60 g PMSG-treated rats (3–8 samples per time-point) (●). The vertical lines indicate ± S.E.M. * P<0.05 compared with untreated group at same time (Scheffé's test). † P<0.05 compared with under-60 g PMSG-treated group at same time (Scheffé's test).
Fig. 4. Changes in plasma GH on day 29 of life in rats weighing under or over 60 g treated with 5 i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Untreated rats (3–11 samples per time-point) (□); over-60 g PMSG-treated rats (4–11 samples per time-point) (○); under-60 g PMSG-treated rats (4–8 samples per time-point) (●). The vertical lines indicate ± S.E.M. * P < 0.05 compared with under-60 g PMSG-treated group at same time (Scheffé’s test).

Fig. 5. Changes in plasma ACTH on day 29 of life in rats weighing under or over 60 g treated with 5 i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Untreated rats (5–11 samples per time-point) (□); over-60 g PMSG-treated rats (5–9 samples per time-point) (○); under-60 g PMSG-treated rats (4–7 samples per time-point) (●). The vertical lines indicate ± S.E.M. * P < 0.05 compared with untreated group at same time (Scheffé’s test). † P < 0.05 compared with same group at 12.00 h (Student’s t-test). ‡ P < 0.05 compared with same group at 10.00 h (Student’s t-test).
Hormonal changes after PMSG treatment

Fig. 6. Changes in plasma oestradiol on day 29 of life in rats weighing under or over 60 g treated with 5 i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Untreated rats (5–9 samples per time-point) (□); over-60 g PMSG-treated rats (7–9 samples per time-point) (○); under-60 g PMSG-treated rats (4–7 samples per time-point) (●). The vertical lines indicate ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001 compared with untreated group at same time (Scheffé’s test). †P < 0.01 compared with same group at 10.00h (Student’s t-test).

Fig. 7. Changes in plasma progesterone on day 29 of life in rats weighing under or over 60 g treated with 5 i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Untreated rats (2–6 samples per time-point) (□); over-60 g PMSG-treated rats (4–11 samples per time-point) (○); under-60 g PMSG-treated rats (4–13 samples per time-point) (●). The vertical lines indicate ± S.E.M. *P < 0.05, **P < 0.005, ***P < 0.001 compared with untreated group at same time (Scheffé’s test). †P < 0.05, ††P < 0.01, †††P < 0.001 compared with same group at 16.00h (Student’s t-test).
Fig. 8. Changes in plasma corticosterone on day 29 of life in rats weighing under or over 60 g treated with 5 i.u. pregnant mare serum gonadotrophin (PMSG) on day 27. Untreated rats (4–10 samples per time-point) (□); over-60 g PMSG-treated rats (3–8 samples per time-point) (○); under-60 g PMSG-treated rats (2–6 samples per time-point) (●). The vertical lines indicate ± S.E.M. * P<0.05, ** P<0.01 compared with untreated group at same time (Scheffé's test), † P<0.05 compared with same group at 12.00 h (Student's t-test).

**Prolactin** (Fig. 2)

Prolactin levels were constant and very low in the untreated animals throughout the afternoon and early evening of day 29. After PMSG treatment prolactin release was stimulated and significantly higher concentrations were seen at all times (except 15.00 h) between 14.00 and 21.00 h in the over-60 g rats. There was, however, no obvious preovulatory peak as has been noted by others (Johnson et al. 1975). In the under-60 g rats prolactin levels were only slightly (and never significantly) higher than those in the untreated animals.

**Thyroid-stimulating hormone** (Fig. 3)

Levels of TSH were constant and remained at approximately 100 µg/l in untreated rats between 13.00 and 21.00 h on day 29. Treatment with PMSG raised the concentrations slightly in both over- and under-60 g rats, reaching significance at 16.00 and 17.00 h for the under-60 g group (P<0.05) and at 17.00 h for the over-60 g rats (P<0.05). The pattern of secretion tended to be different (although not significantly so) in the two groups with higher levels in the afternoon for the under-60 g rats and in the evening for the over-60 g rats.

**Growth hormone** (Fig. 4)

There was a pulsatile release of GH over day 29 in all three groups of rats whether given PMSG or not, with a marked pulse at 17.00 h, although it was not significantly higher than levels noted at other times. However, there was a surge in GH levels at 13.00 h (P<0.01 vs 12.00 h; P<0.02 vs 14.00 h) in the over-60 g PMSG-treated rats, which was seen in two replicate experiments (results were combined for Fig. 4). This rise was not seen in the
under-60 g PMSG-treated rats or the untreated rats, the former group showing a significantly ($P < 0.05$) lower level when compared with the over-60 g group.

**Adrenocorticotropicin (Fig. 5)**

The secretion of ACTH during day 29 was also pulsatile in all three groups, with higher levels in the 16.00–18.00 h period compared with the 10.00–12.00 h period ($P < 0.05$). There was a tendency for ACTH levels to be lowest in the untreated group and highest in the under-60 g PMSG-treated animals, but there was no significant difference at any time between the three groups, except at 16.00 h, when the under-60 g PMSG-treated rats exhibited a significant ($P < 0.05$) surge of ACTH compared with the untreated group.

**Oestradiol (Fig. 6)**

Oestradiol levels were constant at around 0.25 nmol/l at 12.00 h and during the afternoon of day 29 in the untreated animals. Oestrogen secretion was stimulated by PMSG in both the over- and under-60 g rats throughout this period and although levels appeared to be higher in the under-60 g compared with the over-60 g rats, the differences were not significant. The abrupt fall during the afternoon of day 29, noted previously by us and others, did not occur (Wilson et al. 1974; Parker et al. 1976), although in the over-60 g PMSG-treated rats oestradiol concentrations were significantly ($P < 0.01$) lower at 17.00 h compared with 10.00 h.

**Progesterone (Fig. 7)**

Progesterone levels were low throughout the afternoon and evening of day 29 in the untreated animals. Treatment with PMSG induced a significant surge of progesterone starting at 17.00 h and reaching a peak between 20.00 and 21.00 h; this was similar in the over- and under-60 g groups.

**Corticosterone (Fig. 8)**

Untreated and under-60 g PMSG-treated rats had similar low levels of corticosterone and these were constant between 10.00 and 20.00 h of day 29 in the under-60 g PMSG-treated rats, but showed a decline ($P < 0.05$) at 20.00 h compared with the rest of the day in the untreated animals. In the over-60 g rats, PMSG stimulated two surges of corticosterone, one at 13.00 h ($P < 0.01$) and the other at 16.00 h ($P < 0.05$).

**DISCUSSION**

The hormonal changes in untreated rats over the prepubertal and pubertal period have been measured by several groups of workers. The general pattern shown is a rise in gonadotrophins, GH, TSH and oestradiol during the second week of life (Meijs-Roelofs, Uilenbroek, de Jong & Welschen, 1973; Cons. Umezu & Timiras, 1975; Döhler & Wuttke, 1975; Eden, Albertsson-Wikland & Isaksson, 1978) followed from about the beginning of the third week by very low levels of all hormones. Approximately 10–15 days before puberty, i.e. around days 25–26 of life, the following increases in hormone levels have been reported: a rise in LH, which also showed afternoon surges from about 5 days before puberty (Meijs-Roelofs, Kramer & Sander, 1983), a rise in prolactin, showing morning and evening surges (Döhler & Wuttke, 1976; Kimura & Kawakami, 1981), a rise in GH, with a pulsatile pattern of release (Ojeda & Jameson, 1977a; Isaksson, Nutting, Kostyo & Reagan, 1978; Eden, 1979) and a rise in unbound oestradiol (Puig-Duran, Greenstein & MacKinnon, 1979). In addition, progesterone (Morera, Audi, Betrand & Saez, 1978) and corticosterone (Ramaley, 1976) levels rise at this time with the adult circadian pattern of release (Ramaley, 1972, 1978; Ramaley & Bartosik, 1975b); TSH levels remain low over this prepubertal period (Cons et al. 1975). On day 27, therefore, this endocrine resurgence has
only just started and hormone levels are still relatively low compared with those in the late prepubertal or adult female.

Administration of PMSG induces precocious puberty in the early prepubertal rat in that it initiates a pattern of hormonal secretion leading to ovulation which is very similar to that seen just before and during first ovulation in normal puberty (Parker & Mahesh, 1976; Ojeda, Advis & Andrews, 1980). When we found that PMSG treatment only induced ovulation in immature animals weighing more than a critical weight, it seemed that a comparison of the effect of PMSG on animals above and below this weight might elucidate the hormonal changes pertinent to the control of the onset of natural puberty. This seemed especially likely when we found that administration of certain hormones into the under-60 g PMSG-treated rat could induce ovulation.

In a previous paper (ter Haar & Wilson, 1978) we suggested that a different pleiomorphic form of LH exists in the under-60 g rats after PMSG treatment as compared with the over-60 g animals. When we used a heterologous assay, an LH surge was observed in both the PMSG-treated groups in the evening of day 29, but with an homologous assay on the same samples this surge was only noted in the over-60 g group and could not be detected in the under-60 g animals. A pleiomorphic form of FSH may also exist in the under-60 g rats since with S6 antiserum no surge was noted, while in another assay with S1 antiserum a small surge was detected. Since a different set of samples was used for the two assays, however, a pleiomorphic FSH form has not been proven. Pleiomorphs of both the gonadotrophins reacting differently with different antisera have been noted elsewhere in plasma of rats and primates (Peckham, Forster & Knobil, 1977; Wakabayashi, 1977; Chowdhury, Tcholakian & Steinberger, 1980). The biological activities of these forms can be different and their relative amounts alter with changes in steroid milieu (Mukhopadhyay, Leidenberger & Lichtenberg, 1979; Solano, Garcia-Vela, Catt & Dufau, 1980; Minegishi, Igarashi & Wakabayashi, 1981). In the prepubertal rat the FSH form appears to have normal adult activity (Ojeda & Jameson, 1977b), but it is possible that the LH found in our under-60 g rats after PMSG had a different activity from that in the over-60 g and the adult rats and that it is incapable of initiating the changes required for follicular rupture and ovulation. This pleiomorph of LH in the under-60 g rat is not entirely inactive, however, since the preovulatory rise in progesterone, which is known to be under the control of LH (Hillensjo, Bauminger & Ahren, 1976; Goff & Henderson, 1979), still occurs in the under-60 g rats.

Levels of ACTH varied throughout day 29 in all the groups; however, there was a tendency for levels to be lower in the untreated rats compared with the PMSG-treated animals. Levels were higher in the late afternoon/evening hours in all three groups, thus showing a tendency toward the adult circadian pattern of release (Buckingham, Döhler & Wilson, 1978). Interestingly, the ACTH levels in the under-60 g rats tended to be higher (although only significantly so at 16.00 h) than in the over-60 g rats, but in spite of similar (if not higher) levels of ACTH in the under-60 g PMSG-treated group, corticosterone levels in the under-60 g rats were low and similar to those seen in the untreated animals. There was no obvious evening peak of corticosterone as would be expected from the ACTH rhythm, nor was there a midday peak in the untreated rats as noted from day 26 by Ramaley (1976). Pregnant mare serum gonadotrophin stimulates a rise in plasma corticosterone over the midday hours, 2 days after injection (Ramaley & Olson, 1974; Ramaley & Bartosik, 1975a), and this was noted in our over-60 g rats with particularly high concentrations occurring at 13.00 and 15.00 h.

The incongruity of the low concentrations of corticosterone in the presence of normal to high ACTH levels indicates that the adrenals of the under-60 g rats were insensitive to the action of ACTH. This will be investigated in future studies by noting the steroidogenic effect of exogenous ACTH on adrenals of over- and under-60 g rats.

Pregnant mare serum gonadotrophin stimulated oestrogen secretion in both the over- and under-60 g rats and the circulating levels were not significantly different in the two
groups, indicating that the ovarian follicles of both groups had reached the same degree of maturity in response to PMSG and that the lack of ovulatory response in the under-60 g group was probably not due to a defect at the ovarian level. In a previous study we have shown that uterine weights on day 30 in the two groups are similar, indicating similar oestrogenic stimulation (Wilson & Endersby, 1979). In another report (Wilson et al. 1974) we found that the preovulatory oestrogen surge occurs at midday and there is a marked fall in the late afternoon. In the present experiments the fall might have just commenced at 17.00 h; since the gonadotrophin surge occurred rather late (see Fig. 1), oestradiol synthesis might not be terminated as early as in the previous study.

Treatment with PMSG stimulated an increase in prolactin levels during the afternoon of day 29 which was significantly greater in the over-60 g rats compared with the under-60 g rats. Treatment with PMSG also induced a significantly greater pulsatile release of GH in the over-60 g rats at 13.00 h. The higher levels of prolactin and GH in the over-60 g rats may be due to the higher levels of corticosterone in these animals, since corticosterone stimulates prolactin release (Ramaley & Campbell, 1977; Gelato, Meites & Wuttke, 1978) and GH synthesis (Brattin & Portanova, 1979). Alternatively, the higher levels of prolactin in the over-60 g rats may have enhanced corticosterone secretion (Mann, Cost, Jacobson & MacFarland, 1977; Vasquez & Kitay, 1978). Interestingly, the normal levels of oestradiol in the under-60 g rats (compared with the over-60 g group) did not stimulate prolactin secretion sufficiently to produce normal plasma concentrations.

There are shifts in the normal adult circadian rhythm of TSH and thyroid hormone on the day of pro-oestrous so that high levels are seen in the late afternoon as well as in the morning (Brown-Grant, Dutton & ter Haar, 1977; Buckingham et al. 1978). In our animals there was a tendency for the TSH levels to be higher in the late afternoon/early evening in the over-60 g rats, indicating that this pattern is associated with the occurrence of ovulation.

Several reports in the literature have shown that inducing a depletion of either corticosterone, prolactin or GH delays the onset of puberty, indicating that they all exert a positive modulatory effect on this phenomenon (Ramaley, 1976, 1978; Gelato et al. 1978; Ramaley & Phares, 1980; Advis, Richards & Ojeda, 1981; Advis, Smith-White & Ojeda, 1981). In this report we have shown that endogenous levels of these three hormones were all low in the animals that could not be induced to first ovulation by PMSG, and we have also shown in another set of experiments that injection of any one of these hormones allowed PMSG to exert its ovulatory effect (C. A. Wilson, unpublished results). All these hormones may therefore act as fail-safe mechanisms in controlling first ovulation at puberty.

One possibility is that before puberty a form of LH is secreted which has a low activity as far as inducing changes required for ovulation are concerned. The various hormones that advance puberty may do so by inducing a change in pituitary enzyme activity such that the active adult forms of gonadotrophin are produced. An alternative possibility is that these hormones can increase the sensitivity of the ovary toward the low-activity form of LH.

The increase in release of prolactin, GH and corticosterone may occur coincidently with a physical change in the animal such as critical body fat content or metabolic rate or body core temperature (Frisch, 1980; Everard et al. 1983) and these are represented in our rats by a critical body weight.

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Hormonal changes after PMSG treatment


