CONTROL OF PUBERTY IN FEMALE RATS: THE EFFECT OF PTU-INDUCED

HYPOTHYROIDISM AND SYSTEMATIC UNDERNUTRITION⁽³⁶⁾

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SUMMARY

Sprague-Dawley rats were fed Purina Lab Chow with or without propylthiouracil (PTU), 0.001%, 0.01% or 0.1% PTU, ad libitum from weaning to vaginal opening. Mean values for all pubertal measurements are included in Tables 1 and 2. Growth rate (mean±S.E.) was significantly reduced (Neuman-Keuls test; $P < 0.05$ level) in all PTU-fed rats (controls 4.9±0.1 g/day, 0.001% PTU 4.2±0.2 g/day, 0.01% PTU 3.4±0.2 g/day, 0.1% PTU 2.5±0.1 g/day), while age at vaginal opening in rats fed 0.001% PTU (35.8±0.6 days) or 0.01% PTU (36.1±0.9 days) was not significantly different from controls (36.0±0.6 days). Nevertheless, body weight at vaginal opening was lower in rats fed 0.1% PTU (87.6±4.7 g) than in controls (113.6±3.7 g). Pubertal body weight of rats fed 0.1% PTU was also reduced (88.6±3.7 g) but vaginal opening delayed (40.4±0.8 days). Proportions of body fat (6.1-5.1%), protein (15.0-14.1%), and water (72.4-71.3%) at vaginal opening were the same in control and PTU groups. Serum T₄ was greatly diminished and similar in all 3 PTU groups, 0.2-0.3 µg/100 ml, vs 4.8±0.2 µg/100 ml in controls; in rats fed 0.1% and 0.01% PTU, T₃ was 0.9±0.4 ng/100 ml and 0.9±0.6 ng/100 ml, respectively vs 72.6±5.6 ng/100 ml in controls, but not significantly reduced in the 0.001% PTU-fed group (60.7±7.9 ng/100 ml).

In a second experiment, a group of weanling rats (pair-fed) was selected in which each member was fed the daily amount of control diet eaten by a corresponding age- and weight-matched 0.01% PTU-fed rat. During the experiment, both groups maintained the same body weight, growth rate, and food intake, however, only 45% (n=11) of the pair-fed animals had vaginal opening by the time their 0.01% PTU-fed counterparts attained first estrus. Although one of the pair-fed (undernourished) rats attained first estrus, no eggs were found. Despite greatly reduced body weight (105.3±3.5 g vs controls 127.5±6.6 g), growth rate (3.5±0.2 g/day vs controls 5.5±0.1 g/day) and food intake (13.9±0.7 g/100g BW/day vs controls 10.1±0.3 g/100g BW/day), the 0.01% PTU-fed rats exhibited vaginal opening (36.9±0.8 days vs controls 35.6±1 days) and first estrus (39.6±0.9 days vs controls 36.4±1 days) at the usual age. In contrast, pair-fed rats had a lower % fat (4.5±0.1% vs PTU 6.8±0.4%) and higher % protein (16.5±0.3% vs PTU 14.3±0.3%) at the age when 0.01% PTU-fed rats attained first estrus. Serum prolactin levels at first estrus did not differ in rats fed control diet (26.5±12.4 ng/ml) or 0.01% PTU (8.8±1.9 ng/ml), or in pair-fed animals (8.8±4.5 ng/ml) at the age when PTU-fed rats reached first estrus.

SPECULATION

These data show that despite low food intake and retarded growth, female rats made hypothyroid by feeding them PTU during the post-weaning period undergo sexual maturation at the usual age. This is in contrast with the delayed puberty observed in pair-fed (undernourished) animals that attain the same body weight and growth rate. The proportion of fat/lean tissue in the PTU-fed rats is indistinguishable from that in their pubertal controls, but differs greatly in pair-fed animals at that age.

INTRODUCTION

Attainment of a certain "metabolic rate" and/or minimum proportion of fat/lean tissue (7,13,14,20,22) have been proposed as critical factors for the onset of sexual maturation.

In this regard it is pertinent that hypothyroidism in the fetal rat or during the first 4 days of life retards growth, delays sexual maturation, and produces permanent changes in pituitary-hypothalamic function which cannot be duplicated by neonatal food-restriction causing comparably reduced body weight (3, 27). However, when restricted feeding and retarded growth begin after weaning, sexual maturation is delayed (32). Although post-weaning hypothyroidism may also severely retard growth (21), it is not known whether the delay in first ovulation reported for rats that were thyroidectomized after weaning (18) results from alterations in thyroid function per se or from the effects of concomitant growth retardation and reduced food intake.

The present work was undertaken to investigate the effects of post-weaning hypothyroidism and systematic undernutrition on pubertal development in female rats and to evaluate propylthiouracil (PTU)-treated animals as a model for assessing the effect of a hypometabolic state on sexual maturation.

MATERIALS AND METHODS

Experiment I: Effect of increasing doses of PTU on post-weaning female rats.

The dose response curve for PTU was determined by adding PTU to Purina Laboratory Chow (PLC) and feeding the mixture to normal weanling rats. All food mixtures were based upon a single lot of PLC which was used throughout these studies. Dams (Sprague-Dawley, Charles River strain), obtained at a time at which they were pregnant for no more than two weeks were individually caged. Temperature (21°C) and light (14 hours daily) were controlled. At birth, pups were separated from their mothers and male pups discarded. Litters of ten female pups were randomly placed with mothers that had just given birth and had had their pups removed. Mothers were fed PLC and watered ad libitum and individually caged with their pups. Pups were weaned a 21 days of age and placed in individual cages. All weanling rats were divided into groups of 9 or 10 so that the mean weight of each group was not significantly different from that of any other (±3%). Rats in each group were fed either powdered PLC (controls), 0.001% PTU in PLC, 0.01% PTU in PLC, or 0.1% PTU in PLC with water ad libitum. After weaning, each rat was weighed and observed daily for vaginal opening. At 1100 h on the day of vaginal opening rats were weighed, decapitated and blood samples collected. Carcasses were sealed in plastic bags, immediately frozen and stored for carcass analysis as previously described (32). This included oven desiccation, soxhlet extraction by petroleum ether, and a standard micro-Kjeldahl method to determine total body water, fat, and protein, respectively. Serum thyroxine (T₄) was assayed by competitive protein-binding analysis (5) and triiodothyronine (T₃) by radioimmunoassay according to the method of Alexander and Jennings (1).

Experiment II: Comparison of PTU-treated, pair-fed and normal rats.

Female rats (Sprague-Dawley, Charles River strain), weaned at 21 days of age, were separated into 3 groups of 11 animals as described above. Conditions of caging, temperature, water, light and diet preparation were identical to those in Experiment I. Two groups were fed either powdered PLC or 0.01% PTU in PLC and provided with water ad libitum (see results of Experiment I, below). A third, pair-fed, (underfed) group was composed of rats born 5 days after those in the other group. At weaning they were individually matched for weight (±1g) with 21 day old PTU-fed rats. Each day thereafter they were pair-fed the same amount of powdered PLC as that eaten by the PTU-fed animals of the same age 5 days previously. For pair-fed animals this was done by placing the total daily intake into food containers and allowing the animal to consume it during the following 24 hour day. In all instances the 0.01% PTU in PLC mixture was completely eaten, but the elapsed time varied within the group. All food containers were weighed daily to the nearest 0.1 g to determine food intake. Rats were checked daily for both weight (to the nearest 0.1 g) and vaginal opening. At vaginal opening and each following day, vaginal smears were performed until first estrus (completely cornified smear) was attained. At 1100 h on the day of first estrus, the rats were weighed, decapitated, and blood samples taken. Each pair-fed growth control animal was sacrificed at the same age as its pubertal (first estrus) counterpart. Immediately following blood collection the number of tubal ova found in both oviducts was noted. Carcasses were stored and body composition analysis was performed as described above. Serum T₃ and T₄ were assayed by the above methods. Serum prolactin was assayed utilizing the NIAMDD kit and techniques. All samples were assayed in duplicate in the same assay. The intra-assay variation was less than 10%.

Except where stated otherwise, pubertal food intake for both experiments I and II was represented by the average daily amount of food consumed during the 72 hours prior to first estrus or vaginal opening (31). The growth rate for each animal was defined as the mean weight gain per day from weaning to age at sacrifice. The mean growth rate of each group in Experiments I and II was calculated as the average of the individual growth rates for members of that group. The statistical significance of differences between group means was determined by a one way analysis of variance and the Neuman-Keuls multiple range test (34).

RESULTS

Experiment I (PTU dose response curve): Although rats fed 0.001% PTU or 0.01% PTU had reduced growth rates, and the 0.01% PTU animals were significantly lighter than controls at vaginal opening, they showed no difference in age of puberty (Figure 1 and Table 1). Animals fed 0.001% PTU were not statistically lighter than controls at vaginal opening. In the rats fed 0.1% PTU, growth was severely retarded, also their vaginal opening was delayed and occurred at a lower body weight than in controls. There was no difference in proportions of body components at vaginal opening in control animals or those fed PTU. Serum T₄ was significantly reduced and similar in all 3 PTU-fed groups. In the 0.1% and 0.01% PTU-fed groups, levels of serum T₃ were very depressed. Serum T₃ was not significantly reduced in the 0.001% PTU-fed group. Since rats eating food with 0.01% PTU showed a clear decrease in body weight without delaying vaginal opening, 0.01% PTU was selected as the experimental dose for the second experiment.

Experiment II: Although vaginal opening was not delayed, rats fed 0.01% PTU grew at a lower rate, were significantly lighter, and ate less than normal controls at puberty (Table 2). During the 24 hour period prior to vaginal opening, food intake per 100 g of body weight did not differ in normal control (12.6±0.7 g) and PTU-fed groups (11.0±4.1 g). Food intake per 100 g of body weight did, however, differ in normal control (13.4±0.2 g) and PTU-fed groups (10.0±0.2 g) during the 24 hours prior to first estrus. Despite the great reduction in body weight, growth rate, and food intake in the PTU-fed group there was no difference in age and proportion of fat, protein or water between them and the controls at first estrus (Figure 3, Table 2). In addition, the number of tubal ova recovered from PTU-fed rats did not differ from that of controls at first estrus (Table 2). On the other hand, only 5 of the 11 (45%) pair-fed animals attained vaginal opening by the time their analogous PTU-fed counterparts reached first estrus (Figure 2). Of those 5 only 1 showed an estrous smear. No tubal ova were found.

At sacrifice, although growth rate, body weight, and food intake were the same in pair-fed and PTU-fed rats, there were differences in the proportions of body fat and body protein attained by these groups (Figure 3, Table 2). Serum T₃ and T₄ were reduced in the PTU-fed group at first estrus while only T₃ was lowered in the pair-fed rats at sacrifice. There was no significant difference in levels of serum prolactin in any of the groups at sacrifice (Table 2).

Even though all PTU-fed animals in Experiment I and II had biochemical evidence of hypothyroidism and were remarkably small for pubertal rats, they were not otherwise abnormal in appearance.

Discussion

These data show that despite low food intake and retarded growth, female rats made hypothyroid by feeding them PTU during the post-weaning period underwent sexual maturation at the usual age. This is in contrast with the delayed puberty observed in pair-fed (undernourished) animals that attained the same body weight and growth rate.

Sexual maturation is delayed in rats that are undernourished after weaning. Pubertal age in these animals may depend on their physical status since underfed rats grow slowly but do not attain puberty until they weigh at least the same or more than their well-fed, faster growing counterparts (16,31). In some cases pubertal age may be highly correlated with post-weaning growth rate (17).

In Experiment II of the present study, the pair-fed animals had a much lower growth rate and had attained a body weight that was less than that reached by their well-fed controls when the latter reached sexual maturation. As expected, these pair-fed rats exhibited few signs of puberty (Figure 2). This is to be contrasted with the PTU-fed animals which ate the same reduced amount of food and were as underweight as their immature pair-fed counterparts, but had a normal age at sexual maturation. In addition, they also acquired a normal body composition at puberty (Figure 3, Tables 1 and 2). On the other hand, their pair-fed counterparts at that age had lower proportional body fat and an increased percentage of body protein even though they weighed the same. This is characteristic of somatic components in undernourished pubertal rats with delayed and impaired sexual maturation (32) and in adults with persistent diestrus resulting from severe food-restriction and weight loss (unpublished observation). Interestingly, these findings are consistent with those in a recent study of delayed menarche and amenorrhea in ballet dancers. In that study, young women were reported to be extremely lean and were presumed to have a low food intake typical of ballet dancers (15).

In Experiment I, 0.1% PTU-fed rats developed severe hypothyroidism and showed delayed puberty. Proportions of body components were, however, the same as those in the other PTU-fed and control animals at vaginal opening

(Figure 1, Table 1). Our ongoing studies will determine at what stage during development rats attain the body composition found at puberty.

The present study shows that underweight, undernourished hypothyroid animals attain puberty at the normal age and body composition and raises the possible analogy between these findings and the situation in hypothyroid and other hypometabolic children. Girls with long-standing juvenile hypothyroidism occasionally develop precocious puberty when below the body weight at which sexual maturation usually occurs (4). Despite this, proportional body fat in these children is estimated to be in the range normally associated with girls at menarche (7). Precocious puberty has also been observed in an otherwise immature girl who was treated with PTU (26). Further, mentally retarded bed-ridden girls, with a presumed reduction in "metabolic rate", attained menarche at a significantly lower body weight and at an age which was slightly earlier than that of their ambulatory counterparts (24). In this context, the hypothyroid rats were, indeed, sexually precocious in relation to their pair-fed underweight and undernourished controls. In fact, food intake per 100 g of body weight, a correlate of basal metabolism (13,20), was the same in normal control and PTU-fed rats at the day of vaginal opening. Our continuing studies utilizing systematic food-restriction to induce weight loss have shown that underfed and underweight animals cease vaginal cycling (unpublished). It will be interesting to note the effect of hypothyroidism on that model.

Our inability to show elevated prolactin at the time of first estrus in PTU-fed animals is consistent with other studies which show normal or low concentrations in the plasma of adult hypothyroid rats (6,30). These observations seem at variance with reports of girls (2,10) and rats (33) which propose an important role for prolactin in the onset of sexual maturation. It is possible that a substantial portion of biologically active serum prolactin may not be detected by a radioimmunoassay (28). However, biological assays have shown decreases in concentrations of circulating prolactin in adult female rats that were radiothyroidectomized at 25 days of age (21).

The mechanism by which PTU-fed animals attain the usual age and body composition at puberty, despite their retarded growth and undernutrition, is not clear. That PTU may directly effect growth and puberty independent of changes in thyroid function has not been ruled out in this experiment. Direct PTU action, however, seems unlikely since a previously reported preliminary study of untreated thyroidectomized rats (18) showed a pattern of growth and puberty which may be similar to PTU-fed animals. In that study hypothyroidism resulting from thyroidectomy on day 24 appears to have caused a suppression of body weight in developing rats. Vaginal opening occurred at the normal time, but they had a slight delay in first ovulation.

There is little doubt that puberty is more closely related to an individual's growth and associated metabolic and nutritional status than to chronological age (7,13,14,17,20,22,32). Unresolved, however, is whether this status is subservient to increasing hormone levels normally associated with sexual development, or whether some as yet undefined aspect of growth is critical for pubertal onset. In this regard, the attainment of a certain body weight or growth rate may be discounted as invariably necessary since in this experiment and others (3,13,17) they are greatly dissociated from puberty. Even proportional and absolute amounts of body fat have been shown to vary at vaginal opening in rats on different dietary regimens (16). Food intake per 100 g of body weight, although similar at vaginal opening, differed at first estrus in the PTU and control rats.

Finally, in addition to the above, other possible mechanisms which could account for PTU's effect on pubertal development may include alterations in steroid hormone metabolism (8,9,25), elevated levels of circulating gonadotrophins (29), and increased gonadal sensitivity to LH and FSH (17,23).

Regardless of the mechanism by which PTU protects against the delay in sexual maturation in undernourished and underweight subjects, this does not occur without a proportion of fat/lean tissue that is indistinguishable from normal pubertal rats. Although the means by which body composition is linked to the timing of sexual maturation is not clarified by these experiments, that link is seen to more certain and its identification even more compelling (11, 12,14,31,32).

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- The authors wish to thank Mr. Ronald Goldwater, Mrs. Lee Jakko, and Mrs. Bonnie Sargent for their great help throughout these studies.
- A preliminary report of this investigation has appeared in the Program of the 60th Annual Meeting of the Endocrine Society, Miami, Florida, 1978 (Abstract No. 335). This work was supported by a grant from the Medical Research Council of Canada (MTS823).
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- Received for publication, September 16, 1980.
- Accepted for publication, November 25, 1980.

Figure 1. Age and mean body weight of rats fed a control diet or 0.001%, 0.01%, 0.1% PTU diet from weaning (day 21) to vaginal opening (VO) in Experiment I. + indicates the mean age for VO.

Figure 2. Age and mean body weight of rats fed a control or a 0.01% PTU diet or a daily amount of control diet equal in weight to the amount of 0.01% PTU diet eaten each day by their analogous weight-matched PTU fed rats of the same age: VO = Vaginal opening, E = first estrus, + = mean age of VO or E and — = range of ages for E or VO.

Figure 3 Mean (S.E.M.) body weight, and proportions of body protein, fat, and water in control (clear bar) and 0.01% PTU-fed (hatched bar) at first estrus or, in the case of the pair-fed rats (shaded bar), at the age when their weight-matched PTU-fed counterparts attained first estrus. * = Significantly different from control (Neuman-Keul test; $P < 0.05$ level).

Table 1. Experiment I. Mean ($\pm$ S.E.) serum T_3 and T_4 , age, body weight and body composition in control and PTU-fed rats at vaginal opening.

	Control N = 10	0.001% PTU N = 9	0.01% PTU N = 10	0.1% PTU N = 9
T_4 , μ g/100 ml	4.8 ± 0.2	$0.2 \pm 0.1^*$	$0.2 \pm 0.0^*$	$0.3 \pm 0.1^*$
T_3 , ng/100 ml	72.6 ± 5.6	60.7 ± 7.9	$0.9 \pm 0.6^*$	0.9 ± 0.4
Age, days	36.0 ± 0.6	35.8 ± 0.6	36.1 ± 0.9	$40.4 \pm 0.5^*$
Body Weight (BWt), g	113.6 ± 3.7	101.7 ± 4.7	$87.6 \pm 4.7^*$	$86.6 \pm 4.1^*$
Growth Rate, g/day	4.9 ± 0.2	$4.2 \pm 0.2^*$	$3.4 \pm 0.3^*$	$2.5 \pm 0.1^*$
Total Body Water (TBW), g	78.5 ± 2.5	70.8 ± 3.1	$59.6 \pm 3.2^*$	$61.0 \pm 2.6^*$
% TBW	72.4 ± 0.3	72.6 ± 0.1	71.3 ± 0.4	71.5 ± 0.3
Body Fat (BF), g	6.0 ± 0.5	5.4 ± 0.6	5.1 ± 0.4	4.4 ± 0.4
% Fat	5.5 ± 0.4	5.1 ± 0.3	6.1 ± 0.2	5.1 ± 0.3
Body Protein (BP), g	15.3 ± 0.6	14.7 ± 0.8	12.6 ± 0.9	12.6 ± 0.6
% Protein	14.1 ± 0.3	14.4 ± 0.3	15.0 ± 0.4	14.7 ± 0.3

* Significantly different from control (Neuman-Keul test; $P < 0.05$ level)

Table 2. Experiment II. Mean ($\pm$ S.E.) serum T_3 , T_4 and serum prolactin; food intake and body measurements in control, PTU-fed, and pair fed, growth control rats.

	At First Estrus		At Age of First Estrus in 0.01% PTU-fed Counterparts
	Control N = 11	0.01% PTU-fed N = 11	Pair-fed Growth Control N = 11
T_4 (μ g/100 ml)	4.2 ± 0.2	$0.3 \pm 0.0^*$	$3.7 \pm 0.2^+$
T_3 (ng/100 ml)	64.6 ± 2.4	$13.6 \pm 0.4^*$	$47.9 \pm 2.0^+$
Prolactin (ng/ml)	26.5 ± 12.4	$8.8 \pm 1.9^*$	8.8 ± 4.5
Age (days)	36.4 ± 1.0	39.6 ± 0.9	$39.6 \pm 0.9^*$
Body Weight (BWt), g	127.5 ± 6.6	$105.3 \pm 3.5^*$	$102.5 \pm 3.3^*$
Growth Rate, g/day	5.5 ± 0.1	$3.5 \pm 0.2^*$	$3.3 \pm 0.1^*$
Total Body Water (TBW), g	85.7 ± 4.2	$69.9 \pm 2.2^*$	$69.3 \pm 2.0^*$
% TBW	70.9 ± 0.3	70.2 ± 0.4	71.0 ± 0.5
Body Fat, g	7.2 ± 0.6	6.8 ± 0.6	$4.4 \pm 0.6^+*$
% Fat	5.9 ± 0.3	6.8 ± 0.4	$4.5 \pm 0.6^+*$
Body Protein, g	17.1 ± 1.3	14.3 ± 0.5	16.1 ± 0.6
% Protein	14.1 ± 0.3	14.3 ± 0.3	$16.5 \pm 0.3^+*$
Food Intake (FI), g/day	15.5 ± 0.9	$10.2 \pm 0.2^*$	$10.2 \pm 0.2^*$
FI/100g BWt/day, g	13.9 ± 0.7	$10.1 \pm 0.3^*$	10.2 ± 0.3
Number of Tubal Ova	8.6 ± 1.4	6.7 ± 1.2	NONE
	At Vaginal Opening		Pair-fed Growth Control
	Control N = 11	0.01% PTU-fed N = 11	N = 5 (see text)
Age (days)	35.6 ± 1.0	36.9 ± 0.8	37.8 ± 1.8
Body Weight (BWt), g	123.5 ± 6.4	$98.8 \pm 2.9^*$	103.8 ± 4.2
Food Intake (FI), g/day	14.9 ± 0.5	$10.5 \pm 0.3^*$	$11.0 \pm 0.2^*$
FI/100g BWt/day, g	12.6 ± 0.7	11.0 ± 4.1	$9.4 \pm 0.4^+*$

* Significantly different from control and, + significantly different from PTU-fed animals (Neuman-Keuls test; $p < 0.05$ level)