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ORIGINAL PAPER

Regulation of circulating leptin and its soluble receptor during pubertal development in the male rhesus monkey (*Macaca mulatta*)

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Abstract In humans, circulating leptin levels are low in early childhood and rise until puberty, whereas the reverse occurs for the soluble leptin receptor (sOB-R). In women, leptin remains high and sOB-R remains low, but in men leptin declines after adolescence and sOB-R increases. These observations suggest that leptin may regulate the production of sOB-R, and that the increased testosterone in adolescent boys may be responsible for the gender differences in leptin and sOB-R. To test this hypothesis, leptin was administered continuously to agonadal juvenile male monkeys for 16 days. No change in sOB-R was observed. Intact juvenile male monkeys were given pulsatile doses of gonadotropins for a period of 7 weeks to induce precocious puberty and assess the effect on plasma testosterone, leptin, and sOB-R. By 4 weeks testosterone had reached adult levels. No changes were observed in leptin, but by week 4, sOB-R was higher than pretreatment values and remained higher at week 7. These data suggest that leptin may not play a significant role in regulating the production of sOB-R and that gender differences in sOB-R in humans may be driven by the increased production of testosterone at puberty in males.

Keywords Leptin · Leptin receptor · Monkey · Puberty

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Introduction

The soluble form of the leptin receptor (sOB-R) is apparently the major binding protein for leptin in the circulation [1], and the interaction between these circulating proteins probably plays a role in regulating the bioavailability of leptin. At a high molar ratio of sOB-R to leptin, the soluble receptor may compete with membrane-bound leptin receptor in target tissue and thus decrease the bioavailability of the hormone [2, 3]. On the other hand, the soluble receptor may also act as a reservoir for circulating leptin and thus reduce its clearance from the circulation [2, 4].

In man, an inverse relationship between circulating levels of leptin and those of its soluble receptor is a consistent feature of studies examining the effects of gender, age, adiposity, and hormonal status on plasma leptin concentrations. Obese and overweight human subjects have elevated circulating leptin levels and reduced sOB-R levels compared to lean subjects [5, 6]. In obese patients who lost weight due to surgical intervention, leptin levels decreased and sOB-R levels increased compared to pre-operative levels [5, 7]. Obese patients who lost weight after being placed on a low-calorie diet for 3 months showed a similar increase in sOB-R levels and a trend toward decreased leptin values [6]. In normal men, sOB-R levels are negatively correlated with percent fat mass and increase during fasting, whereas circulating leptin levels show an opposite pattern [8]. In males and females alike, sOB-R concentrations are high in the circulation in early childhood, decline through late childhood, and

plateau during puberty and adolescence, whereas circulating levels of leptin are low in early childhood, rise through late childhood, and stabilize during puberty and adolescence [2, 9, 10]. Taken together these observations suggest that leptin negatively regulates the production of its own receptor.

In the post-adolescent period in the human, a gender dichotomy has been observed in the circulating levels of leptin and sOB-R. In post-pubertal females, leptin levels remain high and sOB-R levels remain low. In contrast, in post-adolescent males leptin levels decline initially and remain low in adults while sOB-R levels increase [8, 10]. In the male macaque, however, leptin levels do not appear to fall during the early post-adolescent period [11–13] as has been reported in human males, and in older adult male macaques leptin levels rise with age [12, 14]. In baboons, as in humans, leptin levels are higher in adult females than in adult males [15] and a similar sexual dimorphism appears to be exhibited by the rhesus monkey [16]. Sex differences in circulating levels of sOB-R have not been studied in non-human primates.

In a previous experiment conducted by our laboratories for an unrelated purpose, recombinant human (rhu) leptin was administered to agonadal prepubertal male rhesus monkeys [17] and in another study recombinant monkey (rm) LH and FSH were administered to intact juvenile male monkeys to precociously activate testicular function [18]. These two earlier experiments provided the opportunity to (1) test the hypothesis that leptin negatively regulates circulating sOB-R and (2) examine the influence of a premature pubertal rise of circulating testosterone on concentrations of sOB-R.

Results

Experiment 1: Effect of leptin infusion on the levels of circulating sOB-R

As reported previously [17], within 12 h of initiation of the leptin infusion, circulating leptin concentrations increased from preinfusion levels of 1.6 ± 0.37 ng/ml to reach a plateau of approximately 15–17 ng/ml, which was then sustained throughout the remainder of the treatment period. Plasma levels of sOB-R before, during, and after leptin treatment are shown in Fig. 1. No significant overall differences were detected in receptor concentrations between treatment groups or in receptor concentrations over the course of treatment.

Experiment 2: Effect of initiating precocious puberty on the levels of circulating sOB-R

Figure 2 (top panel) shows the plasma testosterone (T) levels over the treatment period when the individual values in each inter-pulse interval were averaged. Integrated mean plasma T levels increased significantly by week 1 and after 4 weeks of gonadotropin (GTH; rm FSH and LH) treatment had reached adult male values.

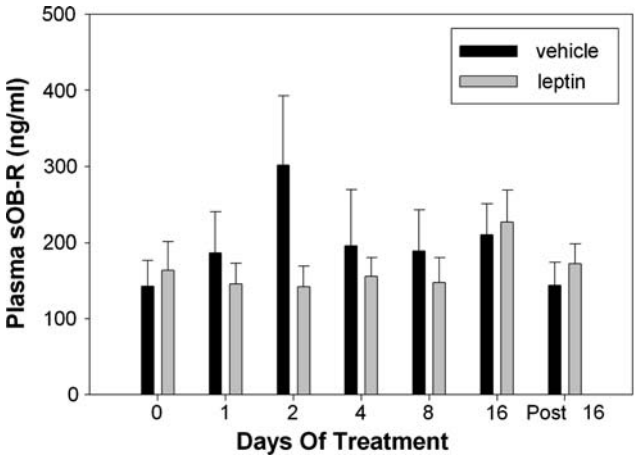

Fig. 1 Mean (±SEM) plasma levels of soluble leptin receptor (sOB-R) in leptin-treated (light bars, 5 μg/kg/h) and control (dark bars) agonadal juvenile male monkeys

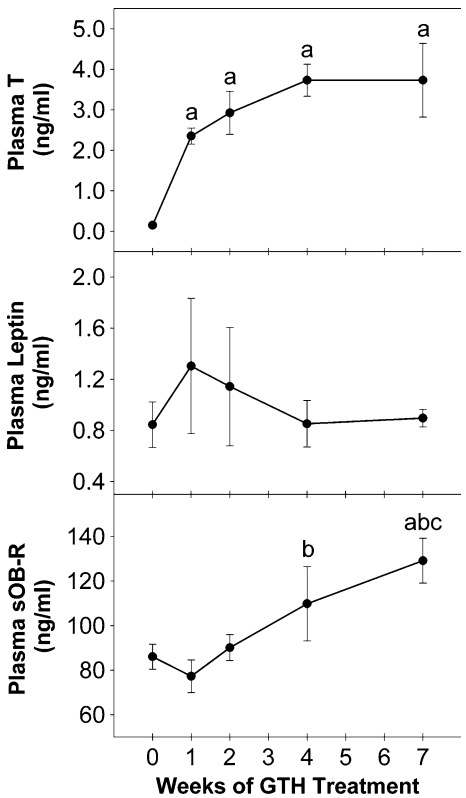

Fig. 2 Changes in mean (±SEM) serum T (top panel), leptin (middle panel), and sOB-R (bottom panel) concentrations in juvenile male monkeys receiving a pulsatile iv infusion of recombinant gonadotropin (FSH and LH doses = 30 and 600 ng/kg/pulse, respectively, every 3 h). a, significantly different from value at week 0; b, significantly different from value at week 1; c, significantly different from value at week 2; $P = 0.05$ or less in all cases

The rise in plasma T concentrations was not associated with any significant change in plasma leptin levels (Fig. 2, middle panel). However, circulating sOB-R concentrations rose significantly ($P < 0.0092$) over the study period, resulting in receptor levels that by week 7 were significantly higher than the levels during pretreatment and at 1 and 2 weeks of treatment (Fig. 2, bottom panel).

Discussion

As reported earlier [17], the infusion of rhu leptin for 16 days to agonadal monkeys resulted in a rapid and sustained 7- to 9-fold elevation in circulating leptin levels. We had previously concluded that this increment in rhu leptin was biologically active in the monkey because of the concomitant increase in growth hormone (GH) secretion. Circulating levels of sOB-R, on the other hand, remained unchanged for the duration of the experiment. This finding is consistent with our earlier study of the infantile male monkey in which sOB-R levels declined over the first year of postnatal life in the face of stable leptin levels [19]. Thus, the relationship between circulating leptin and its receptor in the monkey appears to differ from that in man, in which a negative correlative association has been consistently reported between these circulating proteins [2, 5–10, 20]. In one of those studies [8], Chan and co-workers were able to reverse a fasting induced increase in soluble receptor in men by replacing the associated leptin deficit with recombinant hormone. Moreover in that study, supraphysiological doses of leptin administered to fasted men produced a further decrease in receptor levels. It should be noted, that the endocrine, developmental and nutritional status (gonadally intact, adult and fasted, respectively) of the men in the study by Chan and co-workers [8] were different from those of the monkeys in the current study (agonadal, juvenile, and non-fasted), and the impact, if any, of such experimental differences on leptin's regulation of production of the sOB-R is unknown.

In experiment 2 of the present study, pulsatile GTH administration resulted in a precocious rise in circulating T that reached significance by week 1 of treatment and remained elevated for the rest of the experiment. Changes in circulating leptin levels were not observed over the same period, a finding which is consistent with earlier studies of the relationship between circulating leptin and the rise in testicular T secretion during spontaneous puberty in the monkey [11–13].

In contrast to the unremarkable profile in circulating levels of leptin, sOB-R concentrations increased markedly during GTH treatment. The rise in sOB-R followed that in T, suggesting that the increase in androgen levels may have been responsible for the higher circulating receptor concentrations. This interpretation is compatible with a previous study of male rhesus monkeys, in which circulating sOB-R levels fell in association with decreasing testicular T secretion over the first year of postnatal life [19]. It is also consistent with data we reported for postpubertal human males in which levels of the sOB-R rise post-adolescence in the presence of adult levels of T before plateauing during the fourth decade of life [10]. In contrast, in the human female sOB-R remained constant from the peripubertal period through adulthood and overall levels of this receptor were 3-fold higher in males than females [10]. These gender differences in man support the idea that elevated T levels may be one of the factors responsible for the higher levels of circulating leptin receptor in the human male. In the current study, T levels were elevated by week 1 of GTH treatment but sOB-R levels did not rise significantly until week 4. The time lag time between the observed increase in sOB-R and the rise in T levels suggest that the changes in sOB-R may have resulted from a downstream effect of T that takes longer to develop. This is a question that would be worth addressing in future studies.

In summary, we found that the elevated circulating leptin levels generated by administration of exogenous leptin in agonadal juvenile male monkeys had no effect on circulating levels of sOB-R. However, the precocious pubertal rise in T secretion induced in intact juvenile male monkeys by GTH administration was associated with a significant increase in circulating sOB-R in the absence of a change in circulating leptin. We conclude from these data that: in the male non-human primate leptin does not appear to play a major role in the regulation of its own circulating receptor. In addition, our results suggest that one reason for the gender dichotomy in sOB-R levels observed in human adults may be a testicular T-driven enhancement of sOB-R production.

Materials and methods

Animals

Ten juvenile male rhesus monkeys (*Macaca mulatta*, 14–20 months of age, 2.0–3.5 kg body weight) that were used for two other previously published studies [17, 18] served as the source of blood samples for this report. One group of monkeys was castrated [17]. Animals were housed individually or in pairs under controlled photoperiod (lights on 0700–1900 h) and temperature (20°C) at the Primate Core of the Specialized Cooperative Centers Program in Reproduction Research at the University of Pittsburgh, and fed daily (between 1000 h and 1200 h) a commercial monkey chow diet (Lab Diet 5045; PMI International Inc., Brentwood, MO) supplemented with seeds and fruit.

Drinking water was provided ad libitum. The monkeys were maintained throughout these studies according to the National Institutes of Health Guidelines for Care and Use of Laboratory Animals, and the experimental protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Surgical procedures

Chronic-indwelling venous catheters, which were made from medical grade Silastic brand tubing (Dow Corning, Midland, MI) were implanted into the internal jugular and into the femoral veins. Monkeys were fitted with a jacket and tether and housed in specialized cages that allowed continuous access to the venous circulation without sedation and with minimal restraint. Details of the surgery, postoperative care, and routine maintenance of these and other animals in remote sampling cages have been described previously [17, 18, 21].

The procedure for bilateral orchidectomy and the postoperative care of the castrated animals was described in the original report [17]. Castration was generally performed between 14 and 19 months of age, but in one animal the testes were removed at 4 months of age.

Hormones

Lyophilized rhu leptin was obtained from the National Hormone and Peptide Program (Los Angeles, CA) or R&D Systems (Minneapolis, MN). Rm FSH and LH were obtained from Dr. A. F. Parlow (National Hormone and Peptide Program). Before catheterization, single blood samples (5–10 ml) were collected by femoral venipuncture under ketamine sedation to harvest serum (for use in preparation of hormone infusates). Custom infusates of leptin and those of gonadotropins were prepared for each animal based on the body weight of the monkey as described previously [17, 18].

GnRH was obtained from the Contraceptive Development Branch, National Institutes of Health (Bethesda, MD). A stock solution (1 mg/ml) was prepared as previously described [22]. The GnRH stock was diluted with normal saline to give a working concentration (0.3 μg/ml), and was kept at –20°C until needed.

Experimental protocols

Experiment 1: Effect of leptin infusion on the levels of circulating sOB-R

Agonadal juvenile male monkeys (16–20 months of age, 3.0–3.5 kg body weight) were used in this experiment. Six animals received a continuous iv infusion of rhu leptin (5 μg/kg BW/h) for a period of 16–22 days, beginning on day 1. Three of these animals had previously received vehicle infusions for a period of 16–22 days and served as controls. Prior to the start of the experiment, the pituitaries of all animals were primed with intermittent iv pulses of GnRH (0.1 μg/min for 3 min once each hour) for approximately 3 weeks. This treatment has been shown to enhance the pituitary responsiveness to GnRH [21] and was part of the original study [17], the aim of which was to examine the ability of leptin to induce GnRH release.

A series of sequential nocturnal blood samples (at 20 min intervals) were taken between 2100 h and 2400 h before the infusion was initiated (day 0), on days 1, 2, 4, 8, and 16 of treatment, and after treatment was terminated (day 17 or 23). Blood samples were previously assayed for serum LH and leptin [17], and in the current study the concentrations of soluble leptin receptor were measured. One sample from the 3-h series for each monkey at each sampling day was assayed for sOB-R. The LH and leptin data have been reported previously [17].

Experiment 2: Effect of initiating precocious puberty on the levels of circulating sOB-R

Four intact juvenile male monkeys (13–16 months of age, 2.0–2.6 kg body weight) were used in this experiment. The animals had been implanted with a single, 3-cm long empty Silastic capsule and had served as a control group for a previous study [18]. Animals were treated with iv pulses of GTH every 3 h for a period of 7 weeks. The doses of rm FSH and LH were 30 and 600 ng/kg BW/pulse, respectively. A series of blood samples was collected at –5 and at +5, 20, 40, 60, 80, 120, and 170 min during selected 3 h inter-gonadotropin pulse intervals on week 0 (pre-GTH treatment) and, subsequently on a weekly basis for 7 weeks, between 0900 h and 1200 h. For the purpose of the current study, results from weeks 0 (pre-GTH), 1, 2, 4, and 7 (post-GTH) are presented. All samples were previously assayed for testosterone (T) and LH concentrations [18]. To determine circulating levels of leptin and sOB-R, four of the eight samples from each series (those from times –5, 20, 60, and 170 min) were pooled and samples from the pools were assayed for leptin and sOB-R.

Hormone assays

Plasma leptin levels were measured by RIA using a kit from Linco Research, Inc. (St Charles, MO). The sensitivity of this assay was 0.24 ng/ml. The intra- and inter-assay coefficients of variation for the leptin assays were 8.7% and 9.7%, respectively. Plasma sOB-R levels were measured by a human ELISA kit (DSL, Webster, TX) that was validated for the rhesus monkey (Fig. 3). The sensitivity for this assay

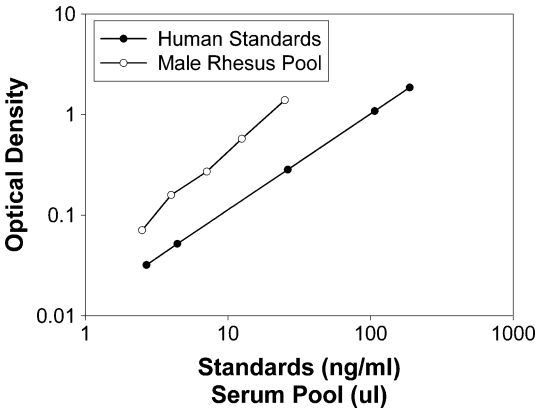

Fig. 3 Validation of the human ELISA for sOB-R in the rhesus monkey. Increasing volumes of a rhesus monkey serum pool produced a dose response that ran parallel to that for the human standard

was 4 ng/ml and the intra- and inter-assay coefficients of variation were 5.6% and 10.5%, respectively. Plasma testosterone (T) levels were measured by either a previously described RIA [23] employing antiserum T3-125 (Endocrine Sciences, Calabasas Hills, CA) or by RIA kit (Total T; Diagnostic Products Corp., Los Angeles, CA). The mean sensitivities of the two T assays were 0.07 and 0.03 ng/ml, respectively. Intra- and inter-assay coefficients of variation for the former method were 9.9% and 9.8%, respectively, and for the kit assay were 9.4% and 7.7%, respectively.

Statistical analysis

The significance of differences between mean values of sOB-R concentrations in experiment 1 was determined by two-way ANOVA (vehicle versus leptin-treatment × time) with repeated measures for time. Additionally, a two-way ANOVA (vehicle versus leptin-treatment × time) with repeated measures for both time and treatment was done just on the data for the three animals that were used in both phases of the experiment (vehicle treatment and leptin treatment), and a two-way ANOVA (animals in both phases versus animals only in leptin-treated phase × time) with repeated measures for time was done just on the data obtained from the leptin-treatment phase of the experiment. The latter two tests were done to detect or rule out any differences that might have occurred due to the fact that some animals participated in both phases of the experiment and some in only the leptin-treated phase of the experiment.

The significance of differences between mean values of hormone concentrations over time in experiment 2 was determined by one-way ANOVA with repeated measures. If an ANOVA detected an overall significant difference, it was followed by the Student's *t*-test for multiple pairwise comparisons between means.

In all cases, a difference at the $P = 0.05$ level or less was considered to be significant. All statistical tests were made using the JMP IN statistical program (version 4.0.4; SAS Institute, Inc., Cary, NC). Hormone concentrations below the sensitivity of the assays were assigned a value equivalent to the minimum detectable concentration.

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