Insulin-like Growth Factor–1 in Syrian Hamsters: Interactions of Photoperiod, Gonadal Steroids, Pinealectomy, and Continuous Melatonin Treatment (43714)

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Abstract. **Four experiments in Syrian hamsters examined the role and possible interaction of photoperiod, gonadal steroids, and the pineal on circulating levels of insulin-like growth factor–1 (IGF-1). In the first experiment, female hamsters were exposed to long photoperiod (LP; 14:10 LD) or short photoperiod (SP; 8:16 LD); an additional group of SP-exposed females was pinealectomized (PX). SP induced a significant depression in IGF-1 concentrations which PX partially prevented. In Experiment 2, two groups (control and castrate [CX]) of adult male hamsters were kept in LP, and three groups (intact, CX, and CX + PX) of hamsters were kept in SP for five weeks. The four groups of animals that were CX and/or maintained in SP had approximately the same mean level of IGF-1, and all four groups were significantly ($P < 0.001$) higher than the LP-control hamsters. In Experiment 3, four groups (intact controls, CX, CX + melatonin pellet [MEL PEL], and MEL PEL only) were kept in LP. Melatonin pellets (1 mg melatonin/24 mg beeswax/every two weeks) were implanted sc twice during the experiment. Castration induced a rise ($P < 0.001$) in IGF-1 levels, and this was not prevented by MEL PEL. In Experiment 4, testosterone and dihydrotestosterone pellets implanted in LP-exposed CX males prevented the CX-induced rise in IGF-1; testosterone implants also reduced IGF-1 levels in CX males treated with progesterone. In conclusion, SP treatment depresses IGF-1 in female hamsters and raises it in males. These results substantiate previous studies in other models of gonadal steroid deficient animals. They lend further credence to the hypothesis that there is a sexual dimorphism in circulating IGF-1 concentrations in the Syrian hamster that may be at least partially related to the presence of gonadal steroids.** [P.S.E.B.M. 1994, Vol 205]

Circulating levels of insulin-like growth factor–1 (IGF-1) are derived mainly from the liver but other extrahepatic tissues including the gonad (1–4) may produce very small amounts. Besides growth hormone (GH), some hormonal factors which may modulate IGF-1 production and release include thyroid and sex hormones and melatonin, a pineal hormone (1–8).

Daily afternoon injections of melatonin induce a sexually dimorphic response in the Syrian hamster by depressing IGF-1 and GH concentrations in female Syrian hamsters (6–8) and raising these hormones in males (8–10). Similarly, light deprivation depressed plasma GH in female hamsters (8), and raised pituitary GH (7) and serum IGF-1 (8) in males at certain times of the day (7). The effects of exposure to short photoperiod (SP) on circulating IGF-1 concentrations in the female hamster is unknown and one of the aims of the present study.

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Besides the effects on GH mentioned above, the best documented sequelae of exposure to SP include its suppression of the neuroendocrine-gonadal axis in male hamsters leading to non-functional gonads and castration-like levels of testosterone; either pinealectomy or implants of a continuous releasing melatonin pellet prevents the gonadal response to SP (11). We hypothesized that if SP raised IGF-1 through its depression of gonadal steroids, then castration (CX) would act like SP in male hamsters and induce a rise in IGF-1 concentrations which could be prevented by replacement treatment with androgens. Further, we examined the alternate proposition that SP might be acting through mechanisms other than suppression of sex steroids and, thus, might act synergistically with CX in raising IGF-1 levels. Finally, we examined the propositions that pinealectomy or continuous melatonin treatment might prevent some of the effects of SP or CX on circulating titers of IGF-1.

Materials and Methods

Young adult animals were purchased from Charles River Canada (St. Constant, Quebec; Experiments 1–3) or from Sasco (Omaha, NE; Experiment 4). Animals were maintained in a windowless vivarium in Edmonton, Canada (Experiments 1–3) or in San Antonio, TX (Experiment 4), and provided with a constant temperature 22° ± 2°C and either a long (LP; 14:10 LD; lights on 06:00 hr) or short (SP; 8:16 LD; lights on 08:00 hr) photoperiod according to the experimental design. All animals received food and water *ad libitum*.

All surgeries (castration, pinealectomy, implants) were performed using rompun (10 mg/kg)–ketamine (100 mg/kg) ip anesthesia (Experiments 1–3) or rodent cocktail (a mixture of ketamine, acepromazine, rompun and NaCl; 1 cc/1.5 kg; Experiment 4) prepared by the veterinarian of the laboratory animal facility. Serum was collected, separated, and stored until radioimmunoassay for IGF-1.

Melatonin, testosterone, dihydrotestosterone, and progesterone were purchased from Sigma Chemical Co. (St. Louis, MO). Beeswax pellets containing 1 mg melatonin, 4 mg testosterone or 4 mg dihydrotestosterone were implanted sc according to the experimental design. Progesterone (50 mg/0.25 cc) was injected sc daily.

Experiment 1. Female hamsters (n = 23) were divided into 3 groups (n = 7–8/group) and exposed to LP or SP for eight weeks. An additional group of SP-exposed animals was PX. Animals were killed between 10:00 and 13:15 h.

Experiment 2. Male hamsters (n = 40) were divided into groups which were exposed to LP or SP. After five days exposure to SP, some SP-exposed animals were further subdivided into groups and PX and/or CX. All animals were killed between 09:00–12:00 hr five weeks later.

Experiment 3. Male hamsters (n = 32) were CX or left intact. These intact or CX hamsters were then divided into groups and implanted with a sc blank beeswax pellet or a MEL PEL (1 mg MEL/24 mg beeswax/every two weeks) for four weeks.

Experiment 4. Male hamsters (n = 48) were CX or left intact and received a daily sc 0.25 ml injection of corn oil or 50 mg progesterone for 10 days. Some groups of CX hamsters concurrently received a blank beeswax pellet or a pellet that contained 4 mg testosterone or 4 mg dihydrotestosterone. All animals were killed between 13:00–15:00 hr.

IGF-1 Assay. All samples were analyzed for IGF-1 by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Before assay, the samples were extracted by acid-ethanol precipitation (87.5% ethanol/12.5% 2 N HCl). Following incubation, the tubes were centrifuged at 4°C for 30 min, the supernatant removed and neutralized by adding 0.855 M Tris-base, and the tubes centrifuged again. The resulting supernatant was diluted with phosphate buffer (pH 7.5) and used in the radioimmunoassay according to the protocol supplied by the vendor.

Statistics. Where appropriate, results were analyzed by a one-way or a two-way analysis of variance followed by a Student-Newman-Keuls test for comparison of multiple means.

Results

Female Syrian hamsters exposed to SP had significantly ($P < 0.05$) lower circulating titers of IGF-1 than the LP-exposed hamsters (Fig. 1). PX tended to reverse the effects of SP by partially restoring IGF-1 titers to control levels (Fig. 1). The gonadal response to SP and its prevention by PX is well documented. The SP-exposed animals in this experiment had small atrophic uteri and suppressed prolactin levels indicative of an adequate time of exposure to SP; PX prevented the effects of SP on the uteri and prolactin levels (data not shown).

In Experiment 2, SP and/or CX induced a significant ($P < 0.001$) rise in circulating concentrations of IGF-1 compared to the LP controls (Fig. 2). There were no significant differences between SP and CX in the IGF-1 levels attained and no effect of PX in preventing the rise caused by SP + CX (Fig. 2). Other hormonal markers were measured to assure that the animals had been exposed sufficiently long to SP (thyroxine, testosterone) and CX (luteinizing hormone) treatment (data not shown).

In Experiment 3, two-way analysis of variance showed that CX caused a significant ($P < 0.001$) rise in circulating IGF-1 concentrations ($P < 0.001$) and both

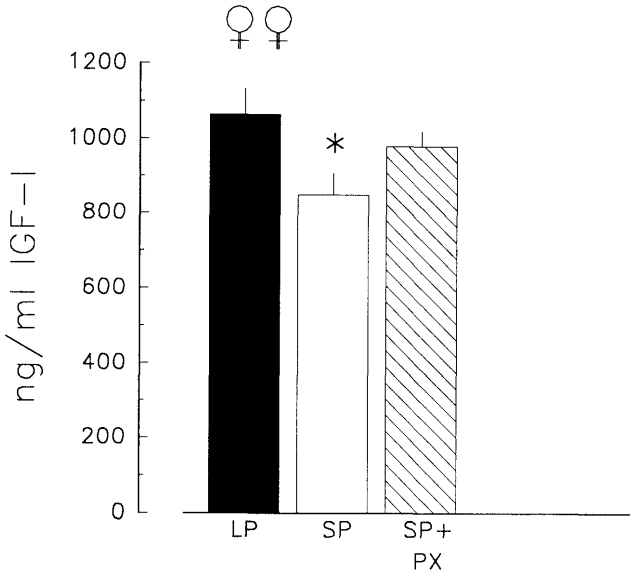

Figure 1. Young adult female Syrian hamsters were exposed to long (LP; 14:10 LD) or short (SP; 8:16 LD) photoperiod for eight weeks. One group of SP-treated hamsters was pinealectomized (PX) at the time of SP exposure. *$P < 0.05$ vs LP control females.

CX and CX + MEL PEL groups had significantly ($P < 0.001$) higher IGF-1 levels compared with the intact controls (Fig. 3). No significant effect of MEL PEL was observed and no significant interaction term between presence or absence of MEL PEL and gonadal status was observed.

In Experiment 4, CX caused a significant ($P < 0.01$) rise in circulating IGF-1 concentrations compared with intact controls. This CX-induced rise was blocked by sc implants of testosterone ($P < 0.025$) or

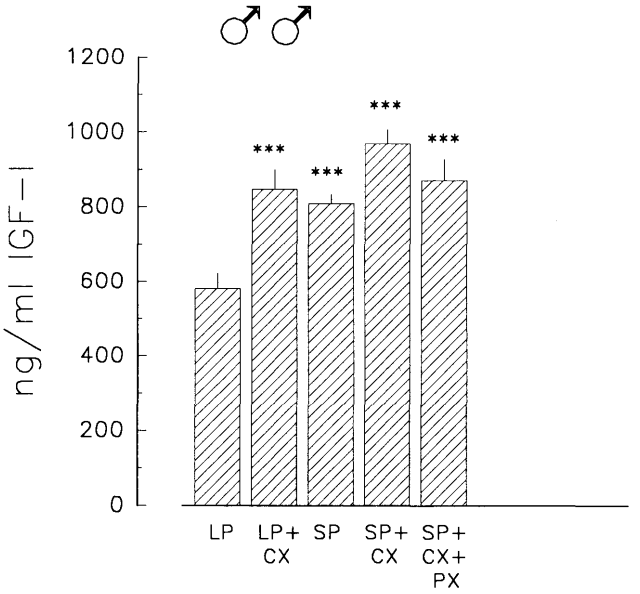

Figure 2. Male Syrian hamsters were maintained in either long (LP; 14:10 LD) or short (SP; 8:16 LD) photoperiod. Some animals in each lighting condition were castrated (CX) and an additional group of SP + CX hamsters was pinealectomized (PX). ***$P < 0.001$ vs LP control males.

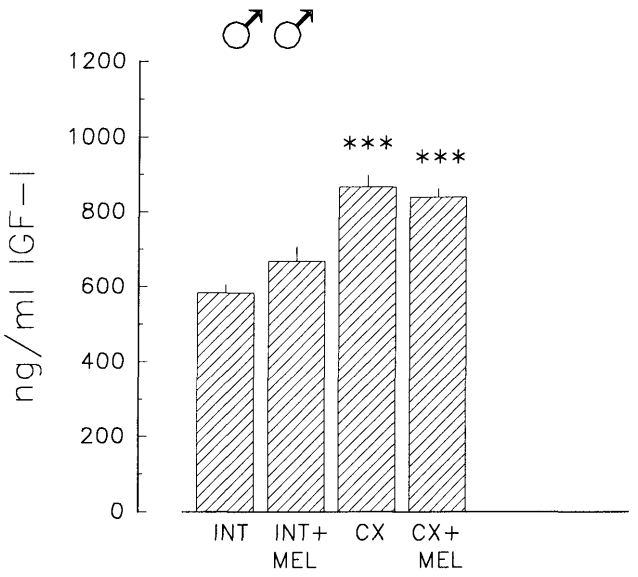

Figure 3. Young adult male Syrian hamsters were kept in long photoperiod (LP; 14:10 LD) for four weeks. Animals were divided into four groups which were either left intact (INT), castrated (CX) and/or received a sc blank beeswax pellet or a melatonin pellet (MEL) containing 1 mg melatonin/24 mg beeswax/every two weeks. ***$P < 0.001$ vs LP controls and LP + MEL pellets. Two-way ANOVA showed a significant effect of CX ($P < 0.001$), no effect of MEL PEL and no significant interaction between surgical treatment and MEL PEL treatment.

dihydrotestosterone ($P < 0.01$). In progesterone treated hamsters, CX did not induce a rise in IGF-1, but implants containing testosterone significantly ($P < 0.05$) suppressed IGF-1 levels compared with the CX group. The values of IGF-1 obtained in this experiment are lower than those observed in the two other experiments involving males. Since different strains were involved we are not sure if this effect is due to strain, age, or some other variable.

Discussion

Experimental treatments which diminish gonadal steroid production in the Syrian hamster (e.g., afternoon melatonin injections, castration) affect serum concentrations of IGF-1 and suggest a modulatory role of steroids on GH and IGF-1 production in this species. Generally, female hamsters have higher IGF-1 concentrations than males and reduction of gonadal steroids tend to reduce IGF-1 levels in females and raise it in males (6–8).

SP treatment is another method by which gonadal steroids are reduced in female hamsters, since the ovaries become enlarged but acyclic while the uterus is small and atrophic. Pinealectomy generally restores ovarian and vaginal cyclicity by preventing most of the actions of SP on the gonad- and thyroid-neuroendocrine axis. Thus, the fall in IGF-1 concentrations induced by SP and its tendency to be reversed by PX in the present experiment are consistent with the notion that PX maintains ovarian cyclicity. One would

predict that injections of female hormones would also restore IGF-1 levels in the female hamster that was castrated, given afternoon melatonin injections, or exposed to SP. Although this has not yet been done in the hamster, experiments involving gonadally deficient primates have shown that injections of estrogens in CX female baboons (12) or hypogonadal girls with Turner's syndrome (13) cause a significant rise in IGF-1 levels.

GH is a well-known mediator of IGF-1 production and secretion. The fall in plasma GH concentrations previously noted in SP-treated females (8) and the reduction in pituitary GH content in females totally deprived of sight (14) are consistent with the depression in IGF-1 seen in SP-treated females in the present experiment. Although the exact interplay of all factors which control IGF-1 secretion in the hamster is not known, previous evidence (6–8) suggests that GH plays a major role in maintaining circulating titers of IGF-1 in this species.

In the present set of three experiments involving male Syrian hamsters, castration of 1.5-, 4- or 5-weeks duration induced a significant rise in IGF-1 levels which was blocked by androgen pellets. However, Meijers and coworkers (15) did not see any change in IGF-1 circulating titers in male hamsters that had been orchiectomized for 16 weeks. There are several reasons which may explain the differences between their results (15) and our set of experiments. Firstly, the effects of castration on IGF-1 levels may diminish over time (five weeks vs. 16 weeks). Secondly, there may be subtle differences in the amount of IGF-1 detected by the biological-radioreceptor assay (15) and the radioimmunoassay although the normal values detected by Meijers *et al.* (15) are equal to those in Figure 4 but somewhat lower than those in Figures 2 and 3. Thirdly, and probably the most important, is that Meijers *et al.* (15) maintained their hamsters under a photoperiod (12 hr light:12 hr dark; Woutersen, personal communication) which led to regressed gonads (1.0221 ± 0.206 g) and presumably low testosterone values. This is a critical issue since the Syrian hamster maintains its gonads in a functional state (normal weight 3–4 g) only if the photoperiod consists of more than 12.5 hr light. Testicular degeneration occurs four to 12 weeks after exposure to a light phase of 12.5 hr or less per 24 hr. Thus, the "intact control hamsters" of Meijers (15) were really exposed to SP for 16 weeks and their "castrate" hamsters were really SP-castrated animals. If that is the case, then the two groups of hamsters in the experiment by Meijers *et al.* (15) are equivalent to two groups of hamsters (SP and SP + CX) in Experiment 2. Based on this, our two experiments are in agreement since we also did not observe any differences between the group exposed only to SP and the SP × CX group.

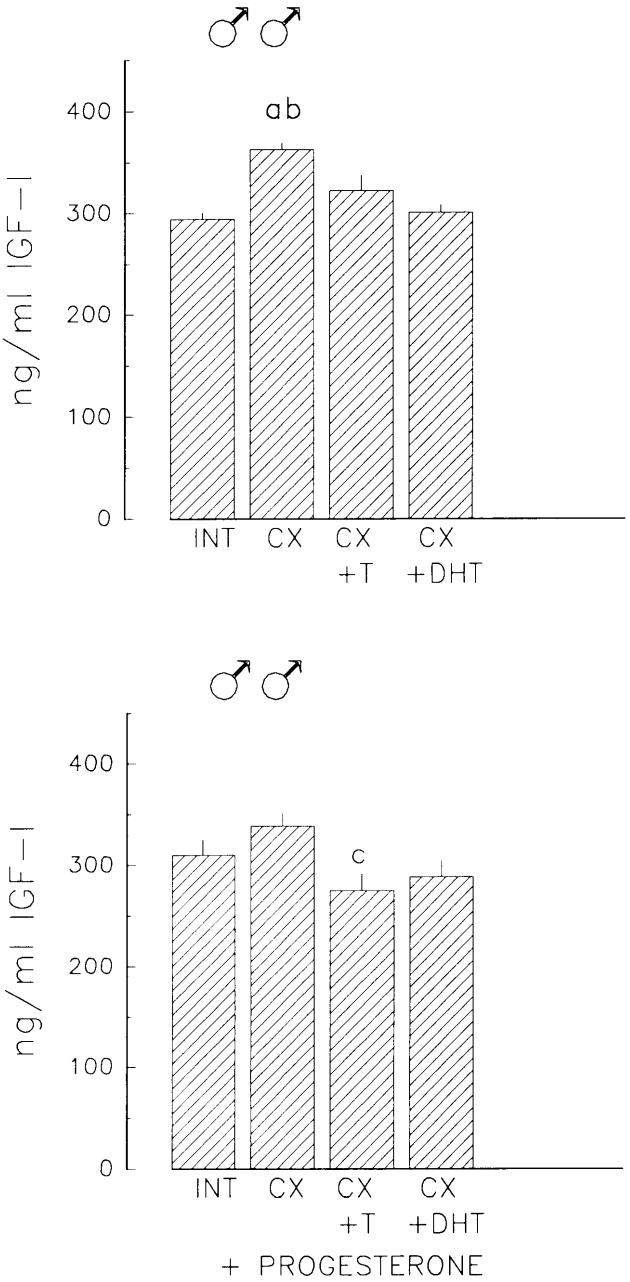

Figure 4. Top panel shows circulating IGF-1 levels in intact (INT) and castrated (CX) male hamsters implanted with pellets containing 4 mg testosterone (T) or dihydrotestosterone (DHT) for 10 days. Bottom panel shows the same groups as in the top panel except that all animals have been treated daily with progesterone. a, $P < 0.01$ vs CON and CX + DHT; b, $P < 0.02$ vs. CX + T.

The observations that continuous MEL treatment did not prevent the rise in IGF-1 in CX hamsters or that PX could not prevent the rise in IGF-1 in SP + CX male hamsters suggests that the gender-related changes in IGF-1 may be dependent almost exclusively on the presence or absence of gonadal steroids and pineal involvement is strictly limited to its role in regulating gonadal steroid status (melatonin injections or SP-treatment being paradigms to produce gonadal-deficient animals). Another possibility is that castra-

tion and SP-treatment are not equivalent models (even though gonadal steroids are depressed to nondetectable levels in both instances) and that castration induces some permanent changes which cannot be overcome by continuous MEL availability or PX.

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1. LeRoith D. In: Insulin-Like Growth Factors. Molecular and Cellular Aspects. Boca Raton: CRC, 1991.
2. Roberts CT Jr., LeRoith D. Interactions in the insulin-like growth factor signaling system. NIPS **7**:69–72, 1992.
3. Handelsman DJ, Spaliviero JA, Scott CD, Baxter JC. Identification of insulin-like growth factor–I/somatomedin C and its receptors in the rat testis. Acta Endocrinol **109**:543–549, 1985.
4. Handelsman DJ, Spaliviero JA, Scott CD, Baxter JC. Hormonal regulation of the prepubertal surge of insulin-like growth factor–I in the rat. Endocrinology **120**:491–496, 1987.
5. Ren SG, Malozowski S, Simoni C, Garcia H, Caruso-Nicoletti M, Cutler GB, Cassorla F. Dose-response relationship between thyroid hormone and growth velocity in cynomolgus monkeys. J Clin Endocrinol Metab **66**:1010–1013, 1988.
6. Vriend J, Sheppard MS, Bala RM. Effects of melatonin and hypothyroidism on somatomedin levels of female Syrian hamsters. J Pineal Res **7**:85–90, 1989.
7. Vaughan MK, Vaughan GM, Little JC, Menendez-Pelaez A, Reiter RJ. Insulin-like growth factor–1 in the Syrian hamster: Circadian rhythm and effects of melatonin. In: Touitou Y, Arendt J, Pevet P, Eds. Int Cong Series 1017: Melatonin and the Pineal Gland—From Basic Science to Clinical Application. Amsterdam: Exerpta Medica, pp289–292, 1993.
8. Vaughan MK, Vaughan GM. Metabolic and thyroidal consequences of melatonin administration in mammals. In: Yu HS, Reifer RJ, Eds. Melatonin: Biosynthesis, Physiological Effects and Clinical Applications. Boca Raton: CRC, pp311–347, 1993.
9. Vriend J, Sheppard MS, Bala RM. Melatonin increases serum insulin-like growth factor–1 in male Syrian hamsters. Endocrinology **122**:2558–2561, 1988.
10. Vriend J, Sheppard MS, Borer KT. Melatonin increases serum growth hormone and insulin-like growth factor I (IGF-I) levels in male Syrian hamsters via hypothalamic neurotransmitters. Growth Devel Aging **54**:165–171, 1990.
11. Reiter RJ, Vaughan MK, King TS, Karasek M. The mammalian pineal gland: Pharmacologic regulation and physiologic consequences. In: Steger RW, Johns AJ, Eds. Handbook of Pharmacologic Methodologies for the Study of the Neuroendocrine System. Boca Raton: CRC, pp331–384, 1985.
12. Copeland KC, Johnson DM, Kuehl TJ, Castracane VD. Estrogen stimulates growth hormone and somatomedin-C in castrate and intact female baboons. J Clin Endocrinol Metab **58**:698–703, 1984.
13. Ross JL, Cassorla FG, Skerda MC, Valk IM, Loriaux DL, Cutler GB. A preliminary study of the effect of estrogen dose on growth in Turner's syndrome. N Engl J Med **309**:1104–1106, 1983.
14. Reiter RJ, Vaughan MK, Vaughan GM, Sorrentino S, Donofrio RJ. The pineal gland as an organ of internal secretion. In: Altschule MD, Ed. Frontiers of Pineal Physiology. Cambridge: MIT Press, pp54–174, 1975.
15. Meijers M, Woutersen RA, van Garderen-Hoetmer A, Bakker GH, de Jong FH, Foekens JA, Klijn JGM. Effects of castration alone, or in combination with aminoglutethimide, on growth of (pre)neoplastic lesions in exocrine pancreas of rats and hamsters. Carcinogenesis **12**:1707–1713, 1991.