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Effects of Pinealectomy and Melatonin or 5-Methoxytryptamine on Testicular LH and PRL Receptors in Syrian Hamsters (*Mesocricetus auratus*)

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A. G. AMADOR, H. G. KLEMCKE, M. K. VAUGHN, A. BARTKE, R. W. STEGER and R. J. REITER. *Effects of Pinealectomy and Melatonin or 5-Methoxytryptamine on Testicular LH and PRL Receptors in Syrian Hamsters* (Mesocricetus auratus). Rev. esp. Fisiol., 44 (1), 81-86, 1988.

The pineal has been previously shown to be an important factor in the regulation of testicular function in photoperiodic mammals. The effects of lack or increase in pineal hormones on testicular hormonal receptors has, therefore, been examined. Pinealectomy decreased the concentration of testicular LH receptors in hamsters exposed to either a long or short photoperiod but had no effect on the concentration of testicular PRL receptors. In animals exposed to a short photoperiod, pinealectomy prevented testicular regression and the concomitant decreases in total LH and PRL receptor contents. Treatment for 12 weeks with either melatonin or 5-methoxytryptamine caused a decrease in testicular PRL receptor levels, whereas the only changes in LH receptor levels were due to melatonin-induced testicular regression. The present results indicate that some of the effects of pineal hormones on the testes are independent of the pineal-induced changes in testes mass and are the consequence of long-term action. Furthermore, testicular function appears to be affected by both the lack or the increase in pineal hormones.

Key words: LH receptors, PRL receptors, Testes, Hamsters, Pineal, Melatonin, Pinealectomy.

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The pineal has been shown to exert an important influence on the lives of most animals (16). This is specially true for animals that are seasonal breeders, and sensitive to photoperiod changes like the Syrian hamster. When adult male hamsters are exposed to a photoperiod less

than 12.5 h of light per day, testicular function is suppressed and testicular regression occurs (7, 9). Short photoperiod-induced testicular atrophy is generally accompanied by decreases in circulating gonadotropin and prolactin levels (3). The reproductive changes depend upon a normally functioning pineal gland, and occur concomitant with increased pineal secretion of serotonin metabolites in the pineal gland; these include melatonin (MEL) and 5-methoxytryptamine (5-MT) (17-19, 25, 30).

Pineal-independent and dependent changes in hypothalamic norepinephrine and dopamine turnovers, and hypothalamic GnRH content are also associated with photoperiod-induced testicular atrophy (27). At the gonadal level, depletion of LH, PRL and FSH receptor levels are among the earliest detectable responses to short photoperiod in Syrian hamsters and the other animals (2, 4, 11, 24, 28). Responses of testicular receptors to altered photoperiods have been attributed in part to changes in plasma LH, PRL, and perhaps FSH (12, 14), but little is known concerning possible involvement of pineal-related substances. Hence, the following experiments were conducted to determine effects of pinealectomy, MEL and 5-MT treatments on testicular LH and PRL receptors in Syrian hamsters.

Materials and Methods

Juvenile (~ 45 days old) male Syrian (Golden) hamsters [Lak;LVG(SYR)], were maintained in a room with controlled temperature (22 ± 2° C) and illumination [14 h light (L): 10 h darkness (D)]. Animals had free access to food and tap water. At the beginning of the experiment hamsters were pinealectomized or sham-operated as described previously (8). After surgery, animals were either placed in a 5L:19D photoperiod or returned to a 14L:10D photoperiod. Twelve weeks after surgery, hamsters were killed, the testes were removed, decapsulated, weighed, placed in polypropylene tubes, rapidly frozen in a dry ice/acetone mixture and stored at −70° C until assayed for LH and PRL receptors.

Another group of juvenile male hamsters maintained in a 14L:10D photoperiod were injected daily at 1600 h with either 15 or 50 μg MEL or 5-MT/animal, or with vehicle (ethanolic-saline, 1:90). After 12 weeks of treatment, the animals were killed, and decapsulated testes were frozen and stored at −70° C until assayed for receptors.

Measurement of LH and PRL receptors was performed using radioreceptor assays as reported previously (12, 13). Specific activities of [^{125}I]iodo-hCG (CR-121; NIH) and [^{125}I]iodo-oPRL (oPRL; NIH-P-S-13) were 67 and 16 μCi/μg, respectively, with respective maximum binding abilities of 44 and 62 %. Protein content of membrane preparations was determined using a modification of the Lowry procedure (15) and BSA as a standard.

Data were analyzed by analysis of variance and the Student-Newman-Keuls multiple range test. The data were first examined for normality of distribution using the Kolmogorov-Smirnov test, and for homogeneity of variance using Barlett's test. Mathematical transformations were made where necessary (23, 26).

Results

Exposure to short-photoperiod (5L: 19D) for 12 weeks produced the expected increase in the concentration and a decrease in the total content of testicular LH receptors (table I a), along with a decrease in the total content of testicular PRL receptors (table I b). Pinealectomy decreased the concentration of LH receptor in hamsters exposed to long-

(14L:10D) or short-photoperiod (5L:19D), below the levels measured in the corresponding sham-operated control animals (Table Ia). In animals exposed to a long-photoperiod, pinealectomy was followed also by a decrease in the content of LH receptors. In contrast, in hamsters exposed to a short-photoperiod LH receptor content was significally greater in pinealectomized than in sham-operated animals (table I a). The effects of pinealectomy on testicular PRL receptors were less dramatic. In hamsters exposed to a short-photoperiod, pinealectomy acted to partly prevent a reduction in PRL receptor content, while in animals exposed to a long-photoperiod, it did not affect either the concentration or content of testicular PRL receptors (table I b). As expected, pinealectomy did not affect testes weight in hamsters exposed to a long-photoperiod but prevented short-photoperiod-induced testicular regression (table II a).

Treatments with MEL or 5-MT did not affect the concentration of testicular LH receptors. However, treatment with the highest dose of MEL used (50 μg/day) caused a significant decrease in total LH receptor content (table III a). Adminis-

Table I. *Effects of pinealectomy on the concentration and total content of testicular LH and PRL receptors in Syrian hamsters.*
Values are mean ± S.E., and groups with similar letter in superscript are not significantly different from each other ($P > 0.05$). Number of hamsters per group are in parentheses.

Treatment	fmol/mg protein	fmol/testes
a) *LH receptors*		
14L:10D+SHAM	4.4 ± 0.3^{b} (8)	157.8 ± 8.2^{z} (8)
14L:10D+PINX	3.0 ± 0.3^{a} (8)	117.1 ± 10.7^{y} (8)
5L:19D+SHAM	8.1 ± 0.8^{c} (8)	37.8 ± 3.9^{x} (7)
5L:19D+PINX	3.3 ± 0.2^{a} (8)	104.9 ± 11.3^{y} (8)
b) *PRL receptors*		
14L:10D+SHAM	21.1 ± 1.5^{a} (8)	1009.7 ± 59.0^{z}(8)
14L:10D+PINX	16.8 ± 1.5^{a} (8)	$831\pm11.48^{y,z}$(8)
5L:19D+SHAM	16.6 ± 1.2^{a} (7)	88.4 ± 8.3^{x}(7)
5L:19D±PINX	16.4 ± 1.6^{a} (8)	682.4 ± 81.7^{y}(8)

SHAM = Sham – operation; PINX = Pinealectomy.

Table II. *Effects on testicular weight of a) pinealectomy (pinx); b) melatonin (MEL) or 5-methoxytryptamine (5-MT), in Syrian hamsters.*
Values are mean ± SE for the number of hamsters indicated. Groups with similar letter in superscript are not significantly different from each other ($P < 0.05$).

Treatment	N	Testicular weight (g)
a)		
14L:10D + SHAM	8	3.21 ± 0.09^{b}
14L:10D + PINX	8	3.11 ± 0.06^{b}
5L:19D + SHAM	7	0.58 ± 0.11^{a}
5L:19D + PINX	8	2.92 ± 0.07^{b}
b)		
14L:10D + Vehicle	8	3.17 ± 0.11^{c}
14L:10D + 15 μg MEL	8	1.85 ± 0.29^{b}
14L:10D + 50 μg MEL	8	0.47 ± 0.26^{a}
14L:10D + 15 μg 5-MT	8	2.94 ± 0.17^{c}
14L:10D + 50 μg 5-MT	8	3.01 ± 0.23^{c}

Table III. *Effects of melatonin (MEL) or 5-methoxytryptamine (5-MT) on the concentration and total content of testicular LH and PRL receptors in Syrian hamsters.*
Values are mean ± SE, and groups with similar letter in superscript are not significantly different from each other ($P > 0.05$). Eight hamsters per group were used.

Treatment	fmol/mg protein	fmol/testes
a) *LH receptors*		
14L:10D+vehicle	3.7 ± 0.8^{a}	127.1 ± 26.8^{y}
14L:10D+15μg MEL	3.6 ± 0.5^{a}	$65.7\pm9.0^{x,y}$
14L:10D+50μg MEL	$5.1+1.3^{a}$	42.6 ± 7.8^{x}
14L:10D+15μg 5-MT	2.3 ± 0.3^{a}	$80.3\pm12.5^{x,y}$
14L:10D+50μg 5-MT	3.0 ± 0.5^{a}	123.9 ± 23.3^{y}
b) *PRL receptors*		
14L:10D+vehicle	22.5 ± 2.9^{b}	923.1 ± 87.3^{z}
14L:10D+15μg MEL	9.7 ± 1.1^{a}	$316.4\pm66.9^{x,y}$
14L:10D+50μg MEL	10.7 ± 1.5^{a}	181.2 ± 43.6^{x}
14L:10D+15μg 5-MT	10.0 ± 1.4^{a}	481.2 ± 78.0^{y}
14L:10D+50μg 5-MT	15.2 ± 1.6^{a}	831.7 ± 127.0^{z}

tration of either dose of MEL (15 μg or 50 μg) decreased both the concentration and the total content of PRL receptors, below levels measured in vehicle-treated hamsters (table III b). Administration of 5-MT decreased the concentration but only the lowest dose decreased the content of PRL receptors. Treatment with MEL caused a significant dose-related decrease in testicular weight whereas treatment with 5-MT did not affect testes weight (table II b).

Discussion

The increase in the concentration and decrease in total content of testicular LH receptors in response to a short photoperiod was expected from earlier studies (1, 28).

Surprisingly, pinealectomy reduced the concentration of LH receptors in the testes regardless of photoperiod. It has been demonstrated that pinealectomy in 14L:10D-housed Syrian hamsters has no effect on plasma LH and FSH concentrations, nor on testicular weight (29). Further, in short-photoperiod-housed hamsters, pinealectomy allows plasma LH, FSH, PRL and testosterone to return to normal levels (18). Hence, in the current studies the observed reduction in concentration and total content of testicular LH receptors in pinealectomized 14L:10D-housed hamsters in the presence of previously demonstrated normal levels of LH, FSH and PRL, suggest involvement of a pineal-derived factor(s) in the regulation of testicular LH receptors, independent of effects mediated by LH, FSH and PRL. It should also be noted that pinealectomy in 5L:19D-housed hamsters restored LH receptor levels to those found in 14L:10D-housed animals, but significantly below those measured in sham-operated controls. Such data again suggest that a pineal-dependent mechanism separate from that which involves LH, FSH and PRL is operable. Since MEL is thought to be one of the chief mediators of short photoperiod-induced testicular quiescence (5, 18, 19, 21), the expected result would have been to find that pinealectomy inhibits the effects of short photoperiod without having any effects in hamsters exposed to long photoperiod. The reversal of the short photoperiod-induced decrease in total content of LH receptors by pinealectomy is almost certainly due to pinealectomy preventing testicular regression in these animals. It shoud also be noted that effects of pinealectomy on testicular weight and testicular LH and PRL receptors, also appear to be mediated by different mechanisms. Weight, but not receptors, remain normal in 5L:19D pinealectomized hamsters; 15 μg 5-MT dramatically depleted testicular PRL receptors, but had no effect on testicular weight; and finally, 15 μg MEL caused a 41 % decrease in testicular weight, but a 56 % and 65 % decrease in testicular PRL receptor concentration and total content respectively.

In the present study, neither pinealectomy nor short photoperiod had any effect on the concentration of PRL receptors in the testes. The significant decrease in the total content of PRL receptors in short-photoperiod exposed hamsters confirms our previous studies (11, 12). Treatment with MEL or 5-MT had no effect on the concentration of LH receptors, while significant changes in LH receptor content were associated with MEL-induced regression of the testes. Moreover, treatment with both MEL or the lowest dose of 5-MT (15 μg/day) caused a significant decrease in both the concentration and the total content of testicular PRL receptors. The higher dose of 5-MT (50 μg/day) decreased significantly only the concentration but not the total content of PRL receptors.

Therefore, the present results indicate that pineal hormones have some effects

on Leydig cell function that occur independently of the previously documented pineal-mediated morphological changes in the male reproductive system (18). If these effects are due to direct action of pineal hormones on Leydig cells, they would be the result of long-term action, since *in vitro* incubation of Syrian hamsters or rat testes in the presence of MEL or other indoleamines did not affect hCG-stimulated steroidogenesis, or LH receptor levels (6, 10). However, since pinealectomy does not alter gonadotropin and prolactin levels in hamsters maintained in long-photoperiods, the present results could be an indication, for direct effects of pineal hormones on testicular function (2, 20, 22). Also it appears that the lack of pineal hormones, and not only their increased secretion, has an effect on Leydig cell function.

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Resumen

Se estudian los efectos del aumento o de la ausencia de hormonas de la pineal sobre los receptores testiculares para hormonas hipofisiarias. La pinealectomía disminuye la concentración de receptores testiculares para LH en hamsters expuestos tanto a un fotoperiodo corto como a uno largo, sin tener ningún efecto sobre la concentración de los receptores testiculares para PRL. En hamsters expuestos a fotoperiodos cortos, la pinealectomía previene la disminución del tamaño de los testículos, así como la reducción de los contenidos totales de receptores para LH y PRL. El tratamiento durante 12 semanas, con melatonina o con 5-metoxitriptamina produce una disminución en los niveles de receptores testiculares para PRL, mientras que los cambios en los niveles de receptores para LH se relacionan con el descenso de peso testicular debido a la melatonina. Los resultados indican que algunos de los efectos de las hormonas pineales sobre los testículos son independientes de los cambios en masa testicular inducidos por la pineal, y serían consecuencia de efectos a largo plazo. Además, la función testicular puede estar aparentemente afectada, tanto por el aumento como por la ausencia de hormonas pineales.

Palabras clave: Receptores de LH, Receptores de PRL, Testículo, Hamster, Pineal, Melatonina, Pinealectomía.

References

1. Amador, A., Bartke, A., Klemcke, H. G., Siler-Khodr, T. M. and Stallings, M. H.: *J. Reprod. Fert.*, 74, 693-700, 1985.
2. Amador, A. G., Richardson, B. A., Klemcke, H. G., Vaughan, M. K., Steger, R. W., Bartke, A. and Reiter, R. J.: *J. Reprod. Fert.*, 78, 557-564, 1986.
3. Bartke, A.: In «The Hamster-Reproduction and Behaviour» (Siegel, H. I., ed.). Plenum Press, New York, 1985, pp. 73-86.
4. Bartke, A., Goldman, B. D., Klemcke, H. G., Bex, F. J. and Amador, A. G.: «In Functional correlates of hormone receptors in reproduction» (Mahesh, V. B., Muldoon, T. G., Saxena, B. B. and Sadler, W. A., eds.). Elsevier/North Holland, New York, 1980, pp. 171-185.
5. Boyd, I. L.: *Biol. Reprod.*, 33, 21-29, 1985.
6. Cutty, G. B., Goldman, B. D., Doherty, P. and Bartke, A.: *Int. J. Androl.*, 4, 281-290, 1981.
7. Gaston, S. and Menaker, M.: *Science*, 158, 925-928, 1967.
8. Hoffman, R. A. and Reiter, R. J.: *Anat. Rec.*, 153, 19-21, 1965.
9. Hoffman, R. A. and Reiter, R. J.: *Science*, 148, 1609-1611, 1965.
10. Jarrigé, J.-F., Thieblot, P. and Boucher, O.: *Acta Endocrinol.*, 107, 117-124, 1984.
11. Klemcke, H. G., Bartke, A. and Borer, K. T.: *Biol. Reprod.*, 29, 605-614, 1983.
12. Klemcke, H. G., Bartke, A. and Borer, K. T.: *Endocrinology*, 114, 594-603, 1984.
13. Klemcke, H. G., Bartke, A. and Goldman, B. D.: *Biol. Reprod.*, 25, 536-548, 1981.
14. Klemcke, H. G., Bartke, A., Steger, R., Hod-

ges, S. and Hogan, M. P.: *Endocrinology*, 118, 773-782, 1986.
15. Markwell, M. K., Haas, S. M., Bieber, L. L., Tolbert, N. E.: *Anal. Biochem.*, 87, 206-210, 1978.
16. Reiter, R. J.: *Am. Zool.*, 10, 189-190, 1970.
17. Reiter, R. J.: *Ann. Rev. Physiol.*, 35, 305-328, 1973.
18. Reiter, R. J.: *Endocr. Rev.*, 1, 109-131, 1980.
19. Reiter, R. J.: In: «The Hamster - Reproduction and Behaviour» (Siegel, H. I., ed.). Plenum Press, New York, 1985, pp. 99-118.
20. Reiter, R. J., Blask, D. E., Johnson, L. Y., Rudeen, P. K., Vaughan, M. and Waring, P. J.: *Neuroendocrinology*, 22, 107-116, 1976.
21. Reiter, R. J., Richardson, B. A., Vaughan, M. K. and Johnson, L. Y.: *Jikeikai Med. J.*, 28 (Suppl. 1), 35-46, 1981.
22. Sackman, J. W., Little, J. C., Rudeen, P. K., Waring, P. J. and Reiter, R. J.: *Horm. Res.*, 8, 84-92, 1977.
23. Schefler, W. C.: Statistics for health professionals. Addison-Wesley, Reading, 1984.
24. Smith, A. J., Mondain-Monval, M., Moller, O. M., Scheller, R. and Hansson, V.: *J. Reprod. Fert.*, 74, 449-458, 1985.
25. Smith, I.: *Psychoneuroendocrinology*, 8, 41-60, 1983.
26. Sokal, R. R. and Rohlf, F. J.: Biometry (2nd), W. H. Freeman and Co., San Francisco, Calif., 1981.
27. Steger, R. W., Reiter, R. J. and Siler-Khodr, T. M.: *Neuroendocrinology*, 38, 158-163, 1984.
28. Tahka, K. M. and Rajaniemi, H.: *J. Reprod. Fert.*, 75, 513-519, 1985.
29. Tamarkin, L., Hollister, C. W., Lefebvre, N. G. and Goldman, B. D.: *Science*, 198, 953-955, 1977.
30. Tamarkin, L., Westrom, W. K., Hamill, A. I. and Goldman, B. D.: *Endocrinology*, 99, 1534-1541, 1976.