REVISTA ESPAÑOLA DE FISIOLOGIA, 49 (3), 175-180, 1993

Deficient Testicular and Adrenal Steroidogenesis in Mutant Cream (e/e) Syrian Hamsters

A. G. Amador*[1], M. Levin[2], U. W. Huck[2], R. D. Hilgers[1] and N. Angel[2]

[1] Department of Obstetrics and Gynecology
SIU School of Medicine
Springfield, IL 62794-9230 (USA) and
[2] Biology Program
Sangamon State University
Springfield, IL 62794-9243 (USA)

(Received on January 15, 1993)

A. G. AMADOR, M. LEVIN, U. W. HUCK, R. D. HILGERS and N. ANGEL. *Deficient Testicular and Adrenal Steroidogenesis in Mutant Cream (e/e) Syrian Hamsters.* Rev. esp. Fisiol., 49 (3), 175-180, 1993.

Coat color genes have been shown to be developmental genes with wide pleiotropic actions. The present study was undertaken to analyze the effects of mutations at the *e* locus of the Syrian hamster on testicular and adrenal steroidogenesis. Although no differences in body weight were detected, cream (e/e) hamsters had larger testes and smaller adrenals than wildtype (+/+) animals. Plasma testosterone levels were lower in *e/e* than in +/+ hamsters. However; testicular progesterone levels were higher, and 17-OH-progesterone and testosterone levels were lower in *e/e* when compared to +/+ hamsters. The efficiency of testicular 17-hydroxylase appear to be reduced in *e/e* hamsters. Adrenal progesterone levels were higher. 17-OH-progesterone, testosterone, dehydroepiandrosterone sulphate and aldosterone levels were similar, and cortisol levels were lower in *e/e* when compared to +/+ hamsters. The efficiencies of adrenal 17-hydroxylase and 17-hydroxysteroid dehydrogenase appear to be reduced in *e/e* hamsters. The present data indicate that steroidogenic deficiencies are present in the testes and adrenals of *e/e* hamsters, and that the gonadal alterations are more severe than the adrenal ones. The *e* locus, in the hamster, could be a developmental gene, or could be coding for a component in a signaling pathway under the control of such a gene.

Key words: Testes, Adrenals, Cream locus, Progesterone, Hydroxyprogesterone, Testosterone, Cortisol, Aldosterone, Steroidogenesis.

* To whom all correspondence should be addressed: Division of Research, Department of OB/GYN, P. O. Box 19230, Springfield, IL 62794-9230 (U.S.A.).

Coat color genes have been shown to be, in their majority, developmental genes with wide pleiotropic actions (7). Among these actions, one finds the regulation of

testicular function (1, 2, 4, 6). Cream Syrian hamsters have a non-extension of eumelanism mutation at the *e* locus. This mutation produces a cream-yellow phenotype, with black eyes. Normally occurring melanism persists in the skin, in the ear pinnea, palpebrae, subcostal glands and in the genital area (10, 11). There appears to be a connection between the metabolic actions of another mutation, dominant spot (*Ds*), and the *e/e* mutant genotype. In *Ds/+* animals there is no reduction of phaeomelanin, but in *Ds/+; e/e* double mutants there is a marked reduction of phaeomelanin, producing a pale straw yellow to off-white coat color (12). This could indicate that the gene product of the *e* locus is located in a metabolic pathway regulated by the gene product of the *Ds* locus: The product of the latter locus is probably either Stem cell factor or its receptor, which are the two most important genes in the regulation of Leydig cell function (1, 6). Also, in mice the equivalent *e* locus has been implicated in interactions, at least in some tissues with the *a* locus, which is also known to have a function in regulating testicular function (2, 14, 16). Therefore the present study was undertaken to analyze the effects of mutations at the *e* locus of the Syrian hamster, on endocrine parameters.

Table I. *Characterization of solid-phase radioimmunoassays.*

Comparison between the statistical parameters of the curves derived from sample pools and those of the standard curves they were assayed with Progesterone (P4), Hydroxyprogesterone (OHP), Testosterone (T), Aldosterone (Ald.). m = slope. Y^1 = Y intercept and r = correlation coeficient.

Hormonal Sample	m	Y^1	r
Testes P4	−1.701	0.391	−1.000
Standard Curve	−1.697	0.394	−1.000
Adrenal P4	−1.759	0.452	−1.000
Standard Curve	−1.755	0.445	−0.999
Plasma OHP	−1.436	−0.447	−1.000
Standard Curve	−1.430	−0.447	−0.999
Testes OHP	−1.502	−0.606	−1.000
Standard Curve	−1.498	−0.607	−0.999
Adrenal OHP	−2.003	1.176	−1.000
Standard Curve	−2.004	1.174	−0.989
Adrenal T	−1.618	0.443	−1.000
Standard Curve	−1.619	0.445	−1.000
Plasma Estradiol	−1.707	3.699	−1.000
Testes Estradiol	curve was flat		
Standard Curve	−1.708	3.703	−0.999
Plasma Cortisol	−1.988	1.266	−1.000
Standard Curve	−1.994	1.263	−0.998
Adrenal Cortisol	−2.028	3.522	−1.000
Standard Curve	−2.028	3.520	−0.997
Adrenal Ald.	−2.234	5.519	−1.000
Standard Curve	−2.235	5.521	−0.989

Materials and Methods

Adult (>12 week old) random bred male cream (Cream/SSU-*e/e*) and normal (Levin/SSU-+/+) Syrian hamsters were bred in the Laboratory Animal Colony at Sangamon State University. The Cream/SSU stock was derived from mutant hamsters that appeared originally in the Levin/SSU colony. Therefore, both stocks have similar genetic backgrounds. All animals were housed in polycarbonate boxes with free access to food and water. The animal room had controlled temperature (22 ± 2 °C) and illumination (14 h light/24 h).

Hamsters were sacrificed (around 10 a.m.) by decapitation, and trunk blood was collected. Plasma was stored at −20 °C until assayed for steroids. Testes were removed, decapsulated and weighed. A 400-500 mg fragment was obtained, and stored frozen at −70 °C. Adrenals were obtained, weighed, and stored frozen at −70 °C. Testes fragments and adrenals were later homogenized in bidistilled water at 10,500 rpm for 1 min, using a Tekmar Tissuemizer. Homogenates were stored frozen at −20 °C until assayed for steroids.

Plasma testicular and adrenal steroid

levels were determined using solid-phase radioimmunoassays (RIA). Since these kits (Diagnostic Product Corporation, Los Angeles, Ca) use standard curves based on human serum, parallelism between each respective standard curve and curves made up by different volumes from pools of each type of sample was determined and confirmed (table I). This had already been determined previously for plasma testosterone, testicular testosterone and plasma progesterone (5).

Data from the RIA were obtained using the RIAPLOT and RIADOSE programs (3). Data were then evaluated using Student's-test (15).

Results

The corporal weight of cream hamsters was similar to that of normal animals (table II). Circulating steroid levels were not significantly different between *e/e* and +/+ hamsters, except for testosterone, the levels of which were lower ($p < 0.001$) in cream hamsters (table II).

Testicular weight was greater in *e/e* than in +/+ hamsters (table III). While the concentration of testicular progesterone was three times higher in *e/e* than in +/+ hamsters, the concentrations of 17-hydroxyprogesterone and testosterone were much lower in cream than in normal hamsters (table III). Testicular 17-hydroxyprogesterone/progesterone and testosterone/progesterone ratios were significantly lower in *e/e* than in +/+ hamsters, while the testosterone/17-hydroxyprogesterone ratio was similar in both types of animals.

Adrenal weight was smaller in *e/e* than in +/+ hamsters (table IV). The adrenal progesterone concentration was greater, and the cortisol concentration was smaller, in *e/e* than in +/+ hamsters. The adrenal concentrations of 17-hydroxyprogesterone, testosterone, dehydroepiandrosterone sulphate and aldosterone were not found to be statistically different between the two types of hamsters (table IV). The adrenal 17-hydroxyprogesterone/progesterone, testosterone/17-hydroxyprogesterone and testosterone/progesterone ratios were significantly lower in *e/e* than in +/+ hamsters. The aldosterone/progesterone and cortisol/17-hydroxyprogesterone ratios were similar in both types of animals.

Table II. *Corporal weight and circulating steroid levels.*
Values are expressed as mean ± sem for the number of animals in parentheses. 17-OH-progesterone (17-OHP).

Parameter	+/+	e/e
Body weight (g)	125 ± 10 (6)	122 ± 3 (4)
Progesterone	3.37 ± 0.88 (6)	2.32 ± 0.63 (4)
17-OHP	282 ± 68 (6)	268 ± 73 (4)
Testosterone	2.65 ± 0.59 (6)	1.13 ± 0.22 (4)
Estradiol	51.3 ± 4.4 (6)	47.7 ± 9.9 (4)
Cortisol	13.2 ± 1.3 (5)	10.6 ± 2.4 (4)

Table III. *Testicular weight and steroid levels.*
Values are expressed as mean ± sem for the number of animals in parentheses. NS = not significat.

Parameter	+/+	e/e	P
Testes weight (g)	2.29 ± 0.52 (6)	2.96 ± 0.10 (4)	< 0.02
Progesterone (P4) (pg/mg)	9.3 ± 2.3 (6)	27.3 ± 8.2 (4)	< 0.01
17-OH-progesterone (OHP) (pg/mg)	20.6 ± 4.7 (6)	12.8 ± 4.2 (4)	< 0.05
Testosterone (T) (pg/mg)	92.6 ± 16.9 (6)	39.4 ± 8.0 (4)	< 0.0005
OHP/P4	2.39 ± 0.67 (5)	0.48 ± 0.12 (4)	< 0.0005
T/OHP	5.19 ± 10.8 (5)	3.99 ± 1.45 (4)	NS
T/P4	11.6 ± 2.6 (5)	1.6 ± 0.4 (4)	< 0.0005

Table IV. *Adrenal weight and steroid levels.*
Values are expressed as mean ± sem for the number of animals in parentheses. NS = not significant.

Parameter	+/+	e/e	P
Adrenal weight (mg)	30.7 ± 2.7 (6)	22.3 ± 2.6 (4)	< 0.002
Progesterone (P4) (ng/mg)	4.41 ± 1.07 (6)	8.58 ± 2.98 (4)	< 0.01
17-OH-progesterone (OHP) (ng/mg)	1.61 ± 0.34 (6)	2.24 ± 0.53 (4)	NS
Testosterone (T) (pg/mg)	30.8 ± 5.9 (6)	24.6 ± 6.6 (4)	NS
Cortisol (F) (ng/mg)	7.84 ± 1.61 (6)	5.15 ± 1.14 (4)	< 0.02
Aldosterone (M) (pg/mg)	78.2 ± 34.1 (5)	92.4 ± 23.5 (4)	NS
DHEA-SO4 (pg/mg)	249.7 ± 124.4 (5)	260.5 ± 70.5 (4)	NS
OHP/P4	0.41 ± 0.10 (6)	0.29 ± 003 (4)	< 0.05
T/OHP	21.1 ± 3.3 (6)	10.8 ± 0.9 (4)	< 0.0005
T/P4	7.95 ± 1.57 (6)	3.08 ± 0.37 (4)	< 0.0005
M/P4	19.9 ± 8.8 (5)	15.3 ± 6.4 (4)	NS
F/OHP	4.34 ± 2.24 (6)	2.48 ± 0.46 (4)	NS

Discussion

It has been previously shown that, in mice and rats, some coat color genes have an important regulatory effect on the endocrine function on the testis (1, 2, 4, 6). Although Syrian hamsters have been used frequently in endocrine research, very little is known about their genetics in general, and much less about the genetic regulation of their endocrine functions. The present set of data shows mutant hamsters that have reduced circulating levels of testosterone. This occurs in spite of *e/e* hamsters having larger gonads. The explanation of this deficiency can be obtained from the tissue steroid levels observed in these animals. Testicular testosterone concentrations were greatly reduced in *e/e* hamsters, while adrenal testosterone was within normal range. This would indicate that the deficiency in circulating levels is a consequence of the reduced testicular production of testosterone. However, the conversion of progesterone to androgens is defective in both the testis and the adrenals of *e/e* hamsters. In the testis the concentration of 17-hydroxyprogesterone is reduced, as is the 17-hydroxyprogesterone/progesterone ratio. Since the testosterone/17-hydroxyprogesterone is not affected, this would tend to indicate that a deficient activity of the testicular 17-hydroxylase in *e/e* hamsters is responsible for the reduction in testicular testosterone. In the adrenals, 17-hydroxyprogesterone and testosterone concentrations are not reduced, compared to those in normal hamsters. Since *e/e* hamsters have higher adrenal progesterone concentrations, one would expect, contrary to the findings, that they would also have higher 17-hydroxyprogesterone and testosterone levels. The paradox can be explained in terms of a deficiency in steroidogenic enzyme activity. Reduced 17-hydroxyprogesterone/progesterone and testosterone/17-hydroxyprogesterone ratios in *e/e* hamsters indicate that there is a deficiency in the activity of the adrenal 17-hydroxysteroid dehydrogenase, and perhaps also of the adrenal 17-hydroxylase. It is obvious from the data, that the adrenal steroidogenic deficiency is far less severe than the one observed in the gonads.

Because it is now clear that the *e* locus has various pleiotropic effects in the hamster, the gene product from the *e* locus cannot be a steroidogenic enzyme. However, it could be either a growth factor, or a component in the signaling path-

way of a growth factor. Indirect evidence for this would be the effect that mutations of the *Ds* locus have on the phenotype of *e/e* hamsters (12). The *Ds* locus most probably codes for a member of the Platelet-derived growth factor or for a member of the family of the receptors for these factors. The most probable members of these families that would be coded for by the *Ds* locus, would be Stem cell factor or its receptor, both of which are responsible for the regulation of gonadal function (1, 6, 8). The signal transduction pathways of these factors are complex, and interact with those of other growth factors like epidermal growth factor (9). Therefore, the product of the *e* locus in Syrian hamsters could be an enzime from that pathway like a phospholipase, or it could be an enzyme that generates second messengers for that pathway like phosphatidylinositol 3'-kinase, or it could be another type of protein involved in the pathway like G-proteins (13).

In conclusion, cream hamsters have deficient steroidogenesis due to a mutation of the *e* locus, which probably codes for a second messenger in a growth factor signaling pathway.

Acknowledgements

We thank Dr. Nada Chang for her useful comments. We thank Mrs. Caroline Lopez for her technical help. We thank the Biology Program, Sangamon State University, for the support to the Syrian hamster colony. These studies were supported by the SIU/OB/GYN Departamental Research Fund (AGA, RDH), and the ReproGen Research Fund (AGA).

Resumen

Se sabe que los genes que determinan el color del pelaje son, en su mayoría, genes encargados del desarrollo y por lo tanto poseen una multitud de funciones. Por esto se estudian los efectos que una mutación en el locus *e* tiene sobre la esteroidogénesis testicular y adrenal de los hámsters sirios. Aunque no se detectaron diferencias en el peso corporal, los hámsters cremas (*e/e*) poseen testículos más grandes, y adrenales más pequeñas, que los hámsters normales (+/+). Los niveles circulantes de testosterona son menores en los hámsters *e/e* que en los +/+. Sin embargo, los niveles testiculares de la progesterona son más elevados y los de la 17-OH-progesterona y los de la testosterona más bajos, en los hámsters *e/e* que en los +/+. La eficiencia de la 17-hidroxilasa testicular aparentemente se encuentra disminuida en los hámsters *e/e*. Los niveles adrenales de progesterona son mayores en los hámsters *e/e* que en los normales, mientras que los del cortisol son menores. No se encuentran diferencias en los niveles adrenales de 17-OH-progesterona, la testosterona, sulfato de dehydroepiandrosterona y aldosterona, entre los dos tipos de hámsters. Aparentemente la eficiencia de la 17-hidroxilasa adrenal y de la 17-hidroxiesteroide deshidrogenasa adrenal están reducidas en los hámsters *e/e*. Se puede concluir que existen deficiencias tanto en la esteroidogénesis testicular como en la adrenal en los hámsters *e/e*, y que las alteraciones testiculares son más severas que las adrenales. Asimismo, se puede proponer que el locus *e* es, probablemente, un gen regulador del desarrollo en el hamster, o quizás el gen responsable de codificar uno de los componentes en la vía de control metabólico a cargo de un gen regulador del tipo anteriormente mencionado.

Palabras clave: Testículos, Suprarrenales, Progesterona, Hidroxiprogesterona, Testosterona, Cortisol, Aldosterona, Esteroidogénesis.

References

1. Amador, A. G., Bartke, A. and Hodges, S. L.: *Genome*, 30 (suppl. 1), 259, 1988.
2. Amador, A. G., Esquifino, A. I., Wolff, G. L. Mayerhofer, A., Steger, R. W. and Bartke, A.: In «Proceedings of the IVth International Congress of Andrology». Monduzzi Editore, Bologna (Italy) 1989, pp. 1-4.

3. Amador, A. G. and Hodges, S. L.: *Comput. Biol. Med.*, 19, 343-354, 1989.
4. Amador, A. G., Klemcke, H. G. and Bartke, A.: *J. Androl.*, 5, 19P, 1984.
5. Amador, A. G., Mayerhofer, A. and Bartke, A.: *Rev. esp. Fisiol.*, 46, 197-204, 1990.
6. Amador, A. G., Parkening, T., Beamer, W., Bartke, A. and Collins, T. J.: *J. Recept. Res.*, 4, 741-753, 1984.
7. Jackson, I. J.: *BioEssays*, 13,439-446, 1991.
8. Motro, B., van der Kooy, D., Rossant, J., Reith, A. and Berstein, A.: *Development*, 113, 1207-1221, 1991.
9. Reith, A. D., Ellis, C., Lyman, S. D., Anderson, D. M., Williams, D. E., Berstein, A. and Pawson, T.: *EMBO J.*, 10, 2451-2459, 1991.
10. Robinson, R.: *Nature*, 176, 353-354, 1955.
11. Robinson, R.: *J. Genet.*, 56, 85-102, 1958.
12. Robinson, R.: In «Handbook of Genetics, Vol. 4: Vertebrates of Genetic Interest», Plenum Press, New York, N. Y., 1979, pp. 261-274.
13. Rottapel, R., Reedijk, M., Williams, D. E., Lyman, S. D., Anderson, D. M., Pawson, T. and Bernstein, A.: *Molec. Cell. Biol.*, 11, 3043-3051, 1991.
14. Silvers, W. K.: The Coat Color of Mice, Springer-Verlag. New York, N.Y., 1979.
15. Sokal, R. R. and Rohlf, F. J.: Biometry (2nd), W. H. Freeman and Co., San Francisco, Ca, 1981.
16. Wolff, G. L., Galbraith, D. B., Domon, O. E. and Row, J. M.: *J. Hered.*, 69, 295-298, 1978.