REVISTA ESPAÑOLA DE FISIOLOGIA, 43, (4), 455-462, 1987

Possible Role of Prolactin in Growth Regulation

A. I. Esquifino*, M. A. Villanúa and C. Agrasal**

Departamento de Bioquímica
Facultad de Medicina
Universidad Complutense de Madrid
28040 Madrid (Spain)

(Received on)

A. I. ESQUIFINO, M. A. VILLANUA and C. AGRASAL. *Possible Role of Prolactin in Growth Regulation.* Rev. esp. Fisiol., 43 (4), 455-462, 1987.

To study the possible role of prolactin on growth regulation pituitary grafted rats of different ages and their sham-operated controls have been used. After the transplant operation of one pituitary gland from a litter-mate donor on day 5 or on day 30 of life, a marked prolactin increase has been observed. This increase has been immediate in 30 day-old rats and delayed in 5 day-old rats in which the elevation starts being significant on day 20 for females and on day 25 for male rats. Pituitary grafting on day 5 of life, with an adult donor gland, resulted in an immediate and marked increase of prolactin values in both sexes. Litter-mate donor pituitary grafting, on day 5 of life, resulted in an increase in body weight that could be directly correlated with the increase in prolactin levels. Adult pituitary grafting resulted in an increased body weight in females with no effects being detected in males. In 30 day-old grafted male and female rats, marked body weight increases were seen, over the whole studied period, together with an increase in nose-tail length (1 cm in female and 1.5 cm in males longer than the control animals). All these changes do not seem not be related to GH modifications in pituitary grafted rats, since GH changes were very slight with a final tendency to lower values in female rats but not in males. All these data could suggest that prolactin might exert a direct effect on growth both in male and female rats. This effect showed sex differences being more marked in females, probably due to additional actions induced by progesterone.

Key words: Prolactin, Hyperprolactinemia, Growth hormone, Growth, Body weight.

Prolactin (PRL) and growth hormone (GH) are generally considered to belong to the same hormonal family since they share a certain number of common biological, immunological and structural features (14, 20-22, 29). These similarities together with recent data showing the existence of mixed GH and PRL secreting cells in pituitary adenomas (3) or in normal pituitary cells under chronic stimuli (11, 13) suggest that both hormones might be involved in the regulation of similar functions and a close relationship seems to emerge.

Growth has been classically related to a normal secretion pattern of the GH-axis.

* To whom correspondence should be addressed.

** Departamento de Fisiología, Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid (Spain).

Persistent increases in GH secretion, leading to achromegaly (5, 12), are associated with hyperprolactinemia in 50% of the cases (16). On the other hand, hyperprolactinemic states are associated with different modifications in GH secretion (7, 15, 16).

To study the possible role of prolactin in growth regulation, pituitary grafted rats have been used. This model has shown previously to be of a great value in the study of prolactin-gonadotropin interrelationships (2, 17, 28).

Materials and Methods

Induction of hyperprolactinemia and general conditions. — Male and female rats, Wistar strain, were kept under controlled conditions of light (12 h light/12 h darkness) and temperature (22 + 1° C). Sanders (Madrid, Spain) rat chow and water was available *ad libitum.* At the age of 5 or 30 days, rats were submitted to a medial laparotomy under intraperitoneal tribromoethanol (0.25 mg/kg weight) anesthesia. After exposing the right kidney a deep pocket was opened between the capsule and the renal parenchyma at the inferior kidney pole. A litter-mate or an adult donor was decapitated, the pituitary gland quickly removed, the neural lobe discarded and the pars distalis grafted in the kidney pocket under the capsule (11, 12). Rats of the same sex and age were sham-operated by just opening the kidney pocket to be used as controls.

Experiment 1. — Groups of 6-8 grafted or sham-operated rats on day 5 of life were decapitated basally (day 5 of life) and at 10, 15, 20, 25, 30, 45 and 60 days of age. Body weight and plasma PRL levels were determined.

Experiment 2. — Groups of 8-10 rats grafted or sham-operated on day 30 of age were decapitated 15, 30 and 60 days after the operation. Body weight and plasma PRL and GH levels were also determined.

Hormone determinations. — Plasma levels of PRL and GH were measured by specific double-antibody radioimmunoassay systems, using materials kindly supplied by NIAMDKK (NIH, Bethesda, MD). Prolactin RIA systems was previously validated in our laboratory (26) and the levels were expressed as μg/l of their specific RP-1 reference preparation. The sensitivity of GH measurements was 0.5 ng/ml of rat GH-RP-1, and the intraassay coefficient of variation was 6%. To avoid the variability of the methods, all samples were measured in the same assay.

Statistics. — Data were analyzed by one-way Anova. Comparison between groups was performed through a Mann-Whytney U test.

Results

Changes in plasma prolactin levels and body weights in male and female rats after grafting one pituitary gland, on day 5 of life or sham-operation are shown in table I and II.

Transplantation of a pituitary gland from a litter-mate donor on day 5 of life (table I, A) resulted in a clear increase ($p < 0.01$) of prolactin levels from day 25 on. No differences were observed in body weights on days 10, 15, 20 or 60 of age but a significant increase ($p < 0.05$) on days 25, 30 and 45 of life was detected.

The grafting of an adult pituitary gland to 5 days-old male rats (table I, B) resulted in a clear increase in plasma prolactin levels from day 10 of age on ($p < 0.01$ for any studied point) without significant differences in body weights, although a tendency to lower values was detected.

Table I. *Effects of pituitary grafting on day five of life on body weight (g) and prolactin levels (µg/l) at different ages after transplant operation in female rats.*
Values are expressed as mean ± sem.

		Age (days) 5	10	15	20	25	30	45	60
Litter mate donors (A)									
Control	PRL	2±0.2	2±0.4	2±0.3	3±0.4	4±2	11±2	11±1.5	23±1
	Body weight	10±0.2	21±2	26±1	41±1	47±1	61±3	129±2	155±1.5
Exptal.	PRL	2±0.2	4±0.2	4±0.4	11±7	25±1	54±8	43±3	70±12
	Body weight	10±0.2	26±1	36±0.7	47±1	65±1	84±1	146±5	170±4
Adult donors (B)									
Control	PRL	2±0.1	3±0.4	3±0.2	3±0.1	4±1	11±2	29±4	23±1
	Body weight	10±0.2	21±0.4	29±1	42±1.3	48±1	60±1.5	122±3	158±3
Exptal.	PRL	2±0.1	55±3	47±3.3	595±100	62±3	64±10	57±3	87±20
	Body weight	10±0.2	22±1	29±0.8	36±1.5	60±2	72±1	143±3.5	174±1.5

Litter-mate donor pituitary grafting on day 5 of life (table II, A) to female rats, resulted in an earlier increase in plasma prolactin levels than in males (day 20 vs 25 of age) and was maintained during the whole period studied. Increased body weights over sham-operated controls was detected in grafted rats from day 20 on.

Adult pituitary transplantation, in 5 day-old female rats was followed by a significance increase in plasma prolactin levels from day 10 of age on. Whereas no differences in body weight were shown on day 10, 15 and 20 of life, a significant increase was observed, from day 25 on, in grafted animals.

Table II. *Effects of pituitary grafting on day five of life on body weight (g) and prolactin levels (µg/l) at different ages after transplant operation in male rats.*
Values are expressed as mean ± sem.

		Age (days) 5	10	15	20	25	30	45	60
Litter mate donors (A)									
Control	PRL	2±0.3	2±0.4	2±0.3	7±2	4±0.8	13±3	19±4	21±2.5
	Body weight	11±0.3	17±1	26±1	47±1	54±5	64±3	126±9	249±7
Exptal.	PRL	2±0.3	4±0.2	4±0.4	11±2	131±11	59±17	52±15	122±37
	Body weight	11±0.3	24±1	32±3	46±1	70±1	92±4	168±4	240±10
Adult donors (B)									
Control	PRL	2.5±0.5	2±0.2	2±0.1	3.5±1.5	10±1.5	15±3	17±4	25±2.5
	Body weight	10.5±0.4	22±0.5	28±0.5	45±0.5	55±2	64±3	129±10	241±8
Expal.	PRL	2.5±0.5	32±7	52±18	56±7	48±10	40±6	55±1	52±4
	Body weight	10.5±0.4	23±1	30±1	40±1.5	58±4	60±1	111±3	253±3

Table III. *Effect of pituitary grafting on day 30 of life on plasma prolactin (PRL) and GH levels (μg/l) and body weight (g) evolution in male and female rats.*
Values are expressed as mean ± sem.

		Age (days) 30	40	60	90
MALE RATS					
	PRL	8 ± 0.5	14 ± 0.5	18 ± 3	21 ± 4
Control	GH	117 ± 29	47 ± 19	27 ± 10	77 ± 12
	Body weight	66 ± 7	130 ± 5	178 ± 7	290 ± 10
	PRL	8 ± 0.5	22 ± 1	125 ± 14	155 ± 15
Exptal.	GH	117 ± 29	20 ± 16	105 ± 14	138 ± 14
	Body weight	66 ± 7	175 ± 4	234 ± 10	346 ± 7
FEMALE RATS					
	PRL	7 ± 0.01	9 ± 0.5	15 ± 1	17 ± 3
Control	GH	36 ± 9	71 ± 10	100 ± 18	86 ± 4
	Body weight	49 ± 3	118 ± 7	151 ± 8	215 ± 3
	PRL	7 ± 0.01	71 ± 8	131 ± 19	139 ± 20
Exptal.	GH	36 ± 9	29 ± 13	42 ± 11	42 ± 11
	Body weight	49 ± 3	133 ± 4	180 ± 10	268 ± 6

In table III, plasma prolactin and GH levels and body weights after the transplant operation can be observed in male and female rats operated at 30 days of age. In both male and female rats, an increase in plasma prolactin levels ($p < 0.01$) and body weights over the whole period studied was observed ($p < 0.05$). An increase in nose-tail lenght in pituitary grafted animals (1 cm in females and 1.5 cm in males) was also seen. Whereas decreased GH levels were detected in female rats on days 15, 30 and 45 after the grafting ($p < 0.05$) an increase in GH levels was found in male rats on days 15, 30 and 60 after grafting ($p < 0.05$).

Discussion

This study seems to confirm that nevertheless the age of the recipient rat, pituitary grafting resulted in an increase in peripheral prolactin values, thus supporting previous data from the literature (2, 4, 6) and from our own group (25). Increased plasma prolactin levels were associated with a significant decrease in the *in situ* pituitary prolactin content (27) suggesting that much of the circulating prolactin was originated in the ectopic pituitary. This suggestion was clearly confirmed in this study considering plasma values found in rats submitted to a pituitary graft on day 5 of life: when a litter-mate donor was used, prolactin increases started on day 20 of life in female and on day 25 of age in male rats, thus suggesting that local regulatory mechanisms need to be developed after grafting in the ectopic pituitary, as has been recently suggested (9, 10). When an adult donor was used a marked increase in prolactin levels was observed immediately after the transplant operation, thus confirming that mature glands were able to release more prolactin to the recipient animals than the immature pituitaries. These different effects on plasma prolactin levels were associated to modifications in the growth pattern as measured by the increase in body weight. In neonatal litter-mate grafted rats, a marked increase in body weight was found starting on the same day in which prolactin was statistically increased over the control group. The same correlation was observed on days 30, 45 and 60 days of life in females, whereas no differences were observed on day 60 of life in males. No effects on body weight were observed in male rats when an adult pituitary was used as donor, whereas in females an increase was shown. These data suggest that the degree of hyperprolactinemia during neonatal life could be responsible for the observed growth changes. In neonatally hyperprolactinemic animals from other ethiologies, altered growth patterns were also shown, without modifications in the GH secretion pattern (7), thus suggesting that prolactin might exert a certain regulatory role on growth rate.

This fact has been also confirmed in hypophysectomized animals treated with prolactin or bearing pituitary grafts which showed a higher growth rate than non-treated animals (8, 24). This effect was more evident in female than in male rats, suggesting a sex-dependent way for prolactin action on body weight. The latter could be confirmed in our data by the fact that whereas increased body weights were observed in female rats, submitted to neonatal pituitary grafting, over the whole period studied, no differences could be detected in male rats.

The effects shown when the transplant operation was performed at 30 days of age, could be due to the well known effects of prolactin increasing food intake (18). This effect might lead to an increase in white fat deposition that could explain the increase in body weight. If this should be the case, no explanation could be given for the increase in body length both in male and female hyperprolactinemic rats. These growth modifications could be related to a direct effect of prolactin, or to an indirect action mediated throgh GH modifications.

After grafting, increased GH levels were shown in males but decreased GH levels were found in female rats. These data could suggest that while in males, prolactin effects on body weight and length was mediated through the observed GH increases, this explanation could not be used for the females, thus seeming to favour the direct effect of prolactin on growth, that should be regarded as sex-dependent. To clarify GH mediation in prolactin effects on growth, further studies need to be done on possible modifications of GH pulsatility in hyperprolactinemic animals.

Pituitary grafted females have been reported to present higher progesterone levels than sham-operated controls, and progesterone has been shown to increase body weight (18). However, this effect could be present only in female rats, thus explaining growth modifications observed in female but not in male rats (18).

A potential direct effect of prolactin could be exerted by inducing a somatomedin-like protein in the liver (23).

All these data could suggest that prolactin might exert a direct effect on growth both in male and female rats. This effect showed sex differences being more marked in females, probably due to additional actions induced by progesterone.

Acknowledgements

This work has been possible through the Grant n.° 371/84 from «Comisión Asesora para la Ciencia y la Tecnología (CAYCIT)», Madrid (Spain). We are indebted to the National Pituitary Program from the National Institute for Health (NIH, Bethesda, Maryland, USA). The tecnhical assistance of Mrs. Krauss and Mrs. Calvo is gratefully acknowledged.

Resumen

Se estudia el papel de la prolactina en la regulación del crecimiento en ratas Wistar macho y hem-

bra de diferentes edades, portadoras de un trasplante hipofisario bajo la cápsula renal. En ratas operadas en el día 5.° de vida se produce un incremento en los niveles de prolactina a partir del día 20 en las hembras y del día 25 en los machos, que se mantiene en ambos casos durante todo el período estudiado. Cuando el trasplante de hipófisis procede de un animal adulto, este incremento se produce el día 10 para ambos sexos. Paralelamente, se produce un incremento en el peso corporal correlacionado al incremento en los niveles de prolactina, en los animales operados en el día 5.° de vida, tanto en machos como en hembras, respecto a sus controles. El trasplante de una adenohipófisis adulta en el día 5.° de vida induce un incremento similar en el peso corporal en hembras que en el caso anterior, mientras que en los machos no se producen modificaciones. Los animales operados en el día 30 muestran un aumento en el peso corporal en ambos sexos que se mantiene en todos los puntos estudiados, así como aumento en la longitud nariz-cola (1 cm en las hembras y 1,5 cm en los machos). Estos resultados no parecen estar relacionados con cambios en la secreción de GH ya que únicamente se encuentran modificaciones muy pequeñas, con una tendencia en la situación crónica a valores más bajos en las hembras y aumentados en los machos. Los resultados sugieren que la prolactina puede ejercer un efecto directo sobre el crecimiento, tanto en machos como en hembras, aunque más marcado en hembras, probablemente a través de efectos producidos por las progesterona.

Palabras clave: Prolactina, Hiperprolactinemia, Hormona de crecimiento, Crecimiento, Peso corporal.

References

1. Adler, R. A.: *Endocr. Rev.*, 7, 302-313, 1986.
2. Bartke, A., Smith, M. S., Michael, S. D., Peron, F. G. and Dalterio, S.: *Endocrinology*, 110, 182-186, 1977.
3. Bassetti, M., Spada, A., Arosio, M., Vallar, L., Brina, M. and Giannattasio, G.: *J. Clin. Endocr. Metab.*, 62, 1093-2000, 1986.
4. Cramer, O. M., Parker, C. R. and Porter, J. C.: *Endocrinology*, 105, 636-640, 1979.
5. Cryer, P. E. and Daughaday, W. H.: *J. Clin. Endoc. Metab.*, 29, 386-393, 1969.
6. Esquifino, A. I.: Tesis Doctoral, Fac. C. Quím. Universidad Autonóma, Madrid, 1979.
7. Esquifino, A. I., Sanchis, R. and Guerri, C.: *Neuroendocrinology*, 44, 483-487, 1986.
8. Esquifino, A. I., Fernández, J. F., Bartke, A., Agrasal, C., Steger, R. and Cebeira, M.: *Neuroendocrinol. Let.*, 9, 5-14, 1987.
9. Fernández, J. F., Cebeira, M., Agrasal, C., Tresguerres, J. A. F., Bartke, A., Esquifino, A. I. and Ramos, J. A.: *J. Endocrinol.*, 113, 45-49, 1987.
10. Fernández, J. F., Ubeda, E., Tresguerres, J. A. F., Esquifino, A. I. and Ramos, J. A.: *IRCS med. Sci.*, 13, 1126-1127, 1985.
11. Fumagalli, G. and Zanini, A.: *J. Cell Biol.*, 100, 2019-2022, 1985.
12. Glick, S. M., Roth, J., Yallow, R. J. and Berson, S. A.: *Rec. Prog. Horm. Res.*, 21, 241-283, 1965.
13. Goluboff, L. G. and Ezrin, C.: *J. Clin. Endoc.*, 29, 1533-1539, 1969.
14. Jibson, M. D. and Li, C. H.: *Int. J. Pept. Protein Res.*, 14, 113-118, 1979.
15. Lamberts S. W. J., Luzzi, A., Chiodini, P. G., Verde, G. and Klijn, J. G. M.: *Eur. J. Clin. Invest.*, 12, 151-156, 1982.
16. Liuzzi, A., Oppizzi, G., Verde, G., Spelta, B., Bonzana, D. and Chiodini, P. G.: In «Neuroendocrinología. Aspectos Básicos y Clínicos» (Martini, L., Motta, M., Oriol-Bosch, A. and Tresguerres, J. A. F., eds.). Salvat, Barcelona (Spain), 1985, pp 369-379.
17. Mena, F., Haiweg, H., and Grosvenor, C. E.: *Endocrinology*, 83, 1359-1362, 1968.
18. Moore, B. J., Gerardo-Gettens, T., Horwitz, B. A. and Stern, J. S.: *Brain Res. Bull.*, 17, 563-569, 1986.
19. Negro-Vilar, A., Ojeda, S., Arimura, A. and McCann, S. M.: *Life Sci.* 1493-1497, 1978.
20. Nicoll, C. S. and Bryant, G. D.: In «Prolactin and Carcinogenesis» (Boyns, A. R. and Griffiths, K., eds). Alpha, Omega, Alpha, Cardiff Wales, 1972, pp 28-38.
21. Nicoll, C. S.: In «Handbook of Physiology» (Knobil, W. H. and Sawyer W. H., eds). American Physiological Society, Washington, USA, 1974 pp 253-292.
22. Nicoll, C. S.: *Perspect. Biol. Med.*, 25, 369-375, 1982.
23. Neill, J.: In «Prolactin. Basic and Clinical correlations», (R. M. MacLeod, M. O. Thorner and U. Scapagnini, eds.), Fidia Research Series. Vol. 1. Liviana Press, Padova (Italy) 1985, pp 522-527.
24. Pfaff, D.: *Proc. 77th Ann. Conv. APA 4*, 211-212, 1969.

25. Tresguerres, J. A. F. and Esquifino, A. I.: *J. Endocrinol.*, 90, 41-51, 1981.
26. Tresguerres, J. A. F., Esquifino, A. I., Pérez-Méndez, L.F. and López-Calderón, A.: *Endocrinology*, 108, 83-87, 1981.
27. Tresguerres, J. A. F., Esquifino, A. I. and Oriol-Bosch, A.: In «Reproductive Processes and Contraception» (K. McKerns, ed.). Plenum Press, New York, 1981, pp 421-447.
28. Tresguerres, J. A. F. and Esquifino, A. I.: *Acta. Physiol. Latinoamer.*, 33, 257-274, 1983.