REVISTA ESPAÑOLA DE FISIOLOGIA, 41, 183-186. 1985

Effects of Pimozide and Domperidone Administration on Prolactin Levels in Neonatally Estrogenized Female Rats

E. Aguilar *, F. López, L. Pinilla, C. Bellido and M. D. Vaticón

Departamento de Fisiología
Facultad de Medicina
Universidad de Córdoba
14080 Córdoba (Spain)

(Received on June 21, 1984)

E. AGUILAR, F. LOPEZ, L. PINILLA, C. BELLIDO and M. D. VATICON. *Effects of Pimozide and Domperidone Administration of Prolactin Levels in Neonatally Estrogenized Female Rats.* Rev. esp. Fisiol., 41, 183-186. 1985.

The effects of two dopaminergic blockers, pimozide and domperidone, on the prolactin secretion were investigated in adult female rats treated neonatally with estrogens (100 μg of estradiol benzoate s.c. on day 1). These rats showed hyperprolactinemia (556 μg/l vs 57.7 in oil-injected) and treatment with pimozide or domperidone failed to increase prolactin levels in the adult age. These results suggest that the hyperprolactinemia in neonatally estrogenized female rats is produced by loss of the dopaminergic inhibition on prolactin secretion, so that the pharmacological blockade of dopaminergic receptors is uneffective. The dopamine levels in hypothalamus were similar in control and estrogenized females suggesting that failure in dopaminergic inhibition is due to a decrease in dopamine secretion to portal vessels.

Key words: Dopaminergic blockers, Neonatal estrogenization, Prolactin.

Prolactin secretion is controlled by a complex system involving multiple factors, with participation of dopamine, serotonine, histamine, epinephrine and other neurotransmitters, being the inhibitory control of dopamine the most important.

The administration of gonadal steroids after birth produced an anovulatory syndrome with hyperprolactinemia (1-3, 13, 14, 16). It has been postulated that the hyperprolactinemia might be a consequence of oestrogenic stimulation (7, 11) acting at pituitary (4) or hypothalamic level (8). Therefore the data shows a lack of correspondence between prolactin and estrogen plasma levels (14), a failure of ovariectomy to affect prolactin levels in androgenized female rats (13) and the persistence of high prolactin levels in the adult ovariectomized neonatally estrogenized rats (2) suggest the participation of other factors in the production of hyperprolactinemia by neonatal steroids exposure. The data showing that neonatal androgen treatment affects permanently

* To whom all correspondence should be addressed.

the tuberoinfundibular dopaminergic (TIDA) system (9) and that bromocryptine treatment decreases prolactin levels in oestrogenized females nearest normal values (3) point out to the possibility that a failure in TIDA system is the principal factor in the production of hyperprolactinemia. To confirm this hypothesis we studied the prolactin response to the administration of two dopaminergic blockers, pimozide and domperidone, in neonatally estrogenized female rats.

Materials and Methods

Female Wistar rats were injected on day one s.c. with 100 μg of estradiol benzoate (Steraloids Inc. Wilton, USA) diluted in 0.1 ml of olive oil. Controls received vehicle only. The animals were maintained under controlled temperature (20°C) and light (12 h light from 7.00 to 19.00) conditions. Vaginal citology was monotorized daily, and only control animals with 4-5 days regular cycles and oestrogenized animals with anovulatory cycles were used. On day 90 the animals were injected with Domperidone (0.1 mg/kg i.p.), pimozide (1 mg/kg s.c.) or saline. Blood samples were obtained before the injections and at 30 and 60 min (in saline and domperidone injected groups) and at 60 and 120 min (in pimozide injected group). All samples were obtained by jugular puncture after light ether anaesthesia, with minor handling and without environmental changes, at the same hour (10.00-12.00) of previous manipulations (vaginal inspection). Blood samples were obtained in 2 min. After centrifugation at 1650×g during 20 min, plasma was separated and frozen at — 20°C until analyzed. Seven to ten rats formed each group.

Prolactin plasma levels were determined by triplicate in a double antibody radioimmunoassay utilizing NIAMDD kits (Bethesda, USA). Rat-Prl-I-4 were labelled with ^{125}I by the chloramine T method (12). Concentrations of the hormone were expressed as μg/l of the reference preparations NIAMDD-rat-Prl-RP-1. The sensitivity of the assay was 0.15 ng/ml. To avoid interassay variation all samples were run in the same assay. The intraassay variability was 9 %.

In one additional experiment neonatally estrogenized female rats (sixteen) and their respective controls (fourteen) were decapitated on day 90, the hypothalamus was dissected, weighed and frozen a — 20°C. The dopamine content was analyzed by a fluorimetric method (17).

Statistical analysis of the results was carried out by the Student's-test.

Results

Prolactin levels were higher ($p<0.01$) in estrogenized (556 ± 55 μg/l) than in control females (58 ± 8). A strong variability in the values of the oestrogenized rats (between 80 and 1 200 μg/l) was observed. Administration of saline does not change the prolactin values either in control or estrogenized animals (data not shown). All control animals responded with an increase in prolactin levels after pimozide administration. The response was maximal 120 min after drug administration. On the other hand, the response in the estrogenized animals was variable. Six animals showed an initial decrease in the prolactin levels whereas the others showed an increase. The decrease was observed in the animals with higher prolactin basal (fig. 1). These data taken together show that prolactin levels are higher ($p < 0.01$) in the estrogenized group 60 min after pimozide administration and similar to control group at 120 min. Both the control and the estrogenized females responded with an increase in prolactin levels to domperidone administration, although the response

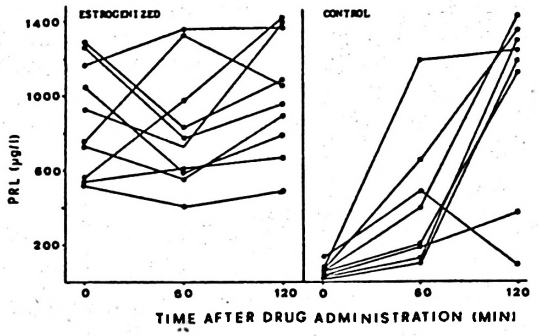

Fig. 1. *Prolactin levels (µg/l) in estrogenized and control female rats at 60 and 120 min after the s.c. administration of pimozide (1 mg/kg).* Each line represents one animal.

was shorter in the estrogenized group (fig. 2). Prolactin levels were similar in control and estrogenized animals 30 min after domperidone administration, and higher ($p < 0.01$) in the controls at 60 min. The response to domperidone or pimozide administration in terms of changes over preinjection levels is shown in figure 3. Both drugs were more effective to induce prolactin level increases in control animals. Dopamine concentration (µg/g) in the hypothalamus was similar in control (0.24 ± 0.01) and estrogenized (0.27 ± 0.02) animals.

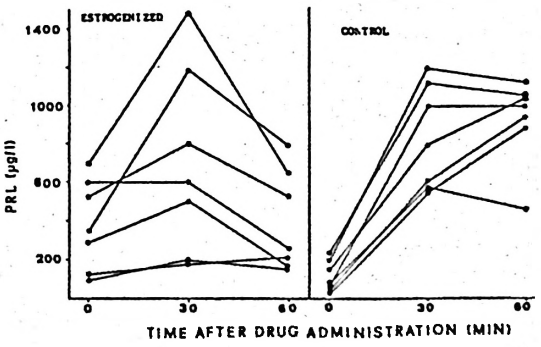

Fig. 2. *Prolactin levels (µg/l) in estrogenized and control female rats at 30 and 60 min after the I.p. administration of domperidone (1 mg/kg).* Each line represents one animal.

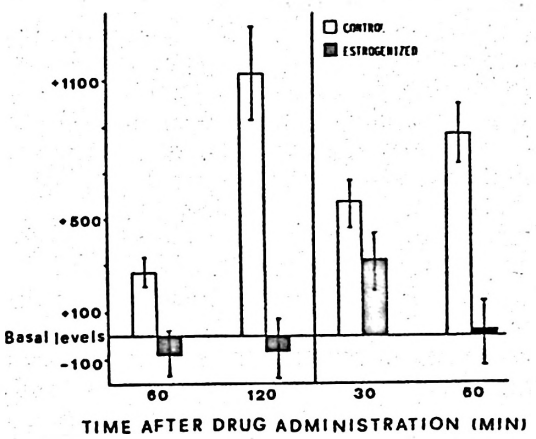

Fig. 3. *Response to pimozide (left) or domperidone (right) administration in control and estrogenized rats.* The response was expressed as changes over preinjection levels.

Discussion

The estrogenized female rats showed hyperprolactinemia in agreement with previous data (1-3, 14). The prolactin levels in estrogenized females showed a great variability suggesting the possibility of different degrees of hypothalamic damage by neonatal steroid exposure.

The blockade of dopaminergic receptors with domperidone or pimozide induced a significant increase in prolactin levels in the control females. In the estrogenized group, the response to pimozide was abolished, and reduced in duration and amplitude after domperidone administration.

Bromocriptine treatment in estrogenized female rats decreased prolactin levels (3), suggesting the maintenance of pituitary response to dopaminergic inhibition. The present findings suggest that neonatal estrogenization produced an attenuation of endogenous dopaminergic inhibition, so that the effectiveness of pharmacological blockade is minimal. Dopamine concentration in the hypothalamus of the estrogenized rats was

normal, so that the failure was probably due to a decrease in dopamine release, in agreement with DEMAREST *et al.* (9) describing changes in dopamine turnover by neonatal androgenization. If this hypothesis was true we could expect a marked decrease in dopamine concentration in the portal vessels of the estrogenized female rats.

It is noticeable that pimozide produced an initial decrease in prolactin levels in estrogenized females with high preinjection values (fig. 1). In some experimental designs an inhibitory action of pimozide, domperidone, haloperidol, chlorpromazine and metoclorpropamide has been previously reported (5, 6, 18). On the other hand, a stimulation of prolactin release by dopamine has been also suggested (10). These «paradoxical» actions may be the consequence of the agonist-antagonist action of dopaminergic blockers (15) or the result of the participation of different dopamine receptors (10).

Acknowledgements

This work has been possible through Grant 0243 from the «Comisión Asesora de Investigación Científica y Técnica» (CAYCIT, Spain). The NIAMDD supplied the radioimmunoassay material for Prolactin determinations.

Resumen

Se estudia en ratas hembras adultas tratadas neonatalmente con estrógenos (inyectadas el primer día de vida con 100 μg de benzoato de estradiol s.c.) el efecto de dos bloqueantes dopaminérgicos, Pimozide y Domperidone, sobre la secreción de prolactina. Los animales estrogenizados presentan en la edad adulta hiperprolactinemia (557 μg/l vs 57,77 de los animales controles) y la administración de pimozide o domperidone no produce incremento de la concentración de prolactina. Estos resultados sugieren que en las ratas hembras estrogenizadas neonatalmente la hiperprolactinemia es debida a una atenuación de la inhibición dopaminérgica, siendo inefectivo el bloqueo de los receptores dopaminérgicos. Los niveles de dopamina en hipotálamo son similares en controles y estrogenizadas, lo que sugiere que la atenuación de la inhibición dopaminérgica es debida a una disminución de la secreción de dopamina a los vasos portales.

References

1. AGUILAR, E., FERNÁNDEZ-GALAZ, C., TEJERO, A. and VATICÓN, M. D.: *Acta Endocrinol.*, suppl. **225**, 177, 1979.
2. AGUILAR, E., FERNÁNDEZ-GALAZ, C., VATICÓN, M. D. and TEJERO, A.: *Acta Endocrinol.*, suppl. **243**. Abstract 352, 1981.
3. AGUILAR, E., FERNÁNDEZ-GALAZ, C., VATICÓN, M. D., TEJERO, A. and ORIOL-BOSCH, A.: *J. Endocrinol.*, **97**, 319-325, 1983.
4. BEN-DAVID, M., DIKSTEIN, S. and SULMAN, F. G.: *Proc. Soc. Exp. Biol. Med.*, **117**, 511-513, 1964.
5. BENTLEY, A. M. and WALLIS, M.: *J. Endocrinol.*, **94**, 317-326, 1982.
6. BESSER, G. M., DELITALA, G., GROSSMAN, A., STUBBS, W. A. and YEO, T.: *Br. J. Pharmac.*, **71**, 569-573, 1980.
7. BISHOP, W., KALRA, C. P., KRULICH, L. and MCCAAN, S. M.: *Endocrinology*, **91**, 1404-1410, 1972.
8. CALIGARIS, L. and TALEISNIK, S.: *Neuroendocrinology*, **21**, 139-145, 1976.
9. DEMAREST, K. T., MCKAY, D. W., RIEGLE, G. D. and MOORE, K. E.: *Neuroendocrinology*, **32**, 108-113, 1981.
10. DENEF, C., MANET, D. and DEWALS, R.: *Nature*, **285**, 243-246, 1980.
11. GALA, R. R.: *Proc. Soc. Exp. Biol. Med.*, **166**, 216-220, 1981.
12. GREENWOOD, F. C., HUNTER, W. M. and GLOWER, J. S.: *Biochem. J.*, **89**, 114-123, 1963.
13. LOOKINGLAND, J. J., WISE, P. P. and BARRACLOUGH, C. A.: *Biol. Reprod.*, **27**, 268-281, 1982.
14. MALLAMPATTI, R. S. and JOHNSON, D. C.: *Neuroendocrinology*, **15**, 255-266, 1974.
15. MCLEOD, R. M. and LAMBERTS, S. W. J.: *Endocrinology*, **103**, 200-203, 1978.
16. RATNER, A. and PEAKE, G. T.: *Proc. Soc. Exp. Biol. Med.*, **146**, 680-683, 1974.
17. SHELLEMBERGER, M. K. and GORDON, J. H.: *Anal. Biochem.*, **39**, 356-372, 1971.
18. WEST, B. and DANNIES, P. S.: *Endocrinology*, **104**, 877-880, 1979.