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Effect of Experimentally Induced Hyperprolactinemia on Growth Hormone Secretion

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A. I. ESQUIFINO, J. J. FERNANDEZ-RUIZ, M. CEBEIRA, C. AGRASAL, J. A. F. TRESGUERRES and J. A. RAMOS. *Effect of Experimentally Induced Hyperprolactinemia on Growth Hormone Secretion.* Rev. esp. Fisiol., 43 (4), 463-468, 1987.

In order to study the existence of possible interrelation-ships between prolactin (PRL) and growth hormone (GH) secretions, adult male rats bearing an anterior pituitary graft under the kidney capsule since day 90 of life and their sham-operated controls were submitted to a single i.p. administration of L-dopa (50 mg/kg weight) or saline 30 days after the operation. Plasma PRL and GH levels were measured by using specific RIA methods. Dopamine (DA) and norepinephrine (NE) contents in the hypothalamus and in the *in situ* anterior pituitary gland were measured by using a specific radioenzymatic assay. An increase in plasma PRL levels and a decrease in plasma GH levels were shown in grafted rats. Hypothalamic contents of DA and NE were increased in these animals, while the anterior pituitary content of DA was not modified as compared to controls. The administration of a single injection of L-dopa led to decreases of plasma PRL and GH levels in both grafted and control rats, but while marked increases in hypothalamic and anterior pituitary contents of DA were shown in both groups, the hypothalamic content of NE was only increased in control animals. These data suggest that PRL and GH secretions were closely related. Dopamine could be mediating the action of PRL on GH, while NE would be less involved.

Key words: Prolactin, Growth hormone, Dopamine, Norepinephrine.

Experimentally induced hyperprolactinemia was associated with different modifications in plasma levels of several pituitary hormones. Decreases of LH (4, 12, 29) and TSH (1), and sex and time dependent modifications of FSH (36) were previously shown. Although some information about possible influences of PRL on GH secretion in humans (2, 19) could be obtained, a complete lack of information about the interrelationships between PRL and GH secretions in other species was found.

Different central neurotransmitters (DA, NE, serotonin, GABA...) have been shown to be involved in the control

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of PRL (23) and GH (20), but a controversy was still maintained concerning GH secretion, which seemed to depend upon the considered sex and species (7, 8, 20, 21, 27, 30, 34). On the other hand, it was well established that hyperprolactinemia acting on the Central Nervous System was able to induce different modifications on hypothalamic neurotransmitter activity (2, 6, 22, 24). These changes could induce an altered GH secretion.

The aim of the present work has been to study the catecholamine mediated possible interrelationships between PRL and GH secretions under hyperprolactinemia.

Materials and Methods

Induction of hyperprolactinemia and sampling. — Adult male rats of the Wistar strain were kept under controlled conditions of light (12 h light/12 h darkness) and temperature (23 ± 1° C). Sanders (Madrid, Spain) rat chow and water were available *ad libitum*. One anterior pituitary gland of a litter-mate female donor was transplanted under the right kidney capsule of each animal at the age of 90 days (37). Rats of the same age were sham-operated to be used as controls. Thirty days after the transplant operation, both control and grafted rats were submitted to a single i.p. injection of L-dopa (50 mg/kg weight) or saline 90 minutes before decapitation. Trunk blood was collected and centrifuged (1,500 *g*) during 5 minutes at 4° C, and the plasma was removed and kept frozen at −20° C until analyzed. The whole hypothalamus and the *in situ* anterior pituitary gland were immediately removed according to GLOWINSKI and IVERSEN (17). Tissues were weighed and homogenized in 50-100 volumes of 0.1N perchloric acid with 1.3 mM EGTA. Homogenates were centrifuged (1500 *g*) during 5 minutes at 4° C and the supernatant fraction was frozen at −20° C until analyzed.

Hormone determinations. — Plasma PRL and GH levels were measured by specific double-antibody radioimmunoassay systems using materials kindly supplied by the National Hormone and Pituitary Program (NIH, Bethesda, Md, USA) and previously validated in our laboratory. The variations of PRL assay have been elsewhere described (35). Plasma PRL levels were expressed as μg/l of rat-PRL-RP-1. Plasma GH levels were expressed as μg/l of rat-GH-RP-1. The intraassay variation was 6% while the interassay variation was 10%.

Assay of dopamine and norepinephrine. — Dopamine and NE contents of removed tissues were measured by a radioenzymatic method according to DA PRADA and ZURCHER (11), previously validated in our laboratory. The variability of the method was earlier described (13). Results were expressed as ng of catecholamine *per* mg of protein measured by the Lowry's method (25).

Statistics. — Data were analyzed by Student's t-test.

Results

Plasma PRL and GH levels after L-dopa or saline administration to both grafted and control rats can be seen in figure 1. One month after the grafting, an increase in plasma PRL levels of grafted *versus* control animals was shown, along with a reduction in plasma GH levels. L-dopa administration resulted in a reduction of both plasma PRL and GH levels, not only in grafted but also in control rats.

One month after the transplant operation, an increase in hypothalamic DA content in grafted as compared to sham-operated animals was observed (fig. 2). A further increase was observed in hypothalamic DA content after L-dopa adminis-

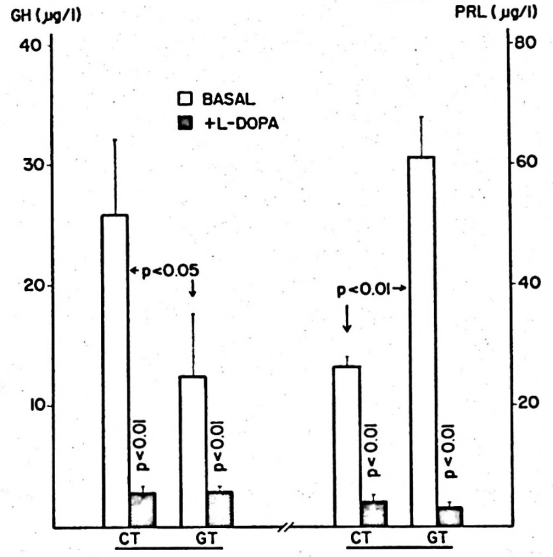

Fig. 1. *Plasma prolactin (PRL) and growth hormone (GH) levels, both basally and 90 min after L-dopa administration (50 mg/kg weight), in grafted (GT) and control (CT) animals.*
Values are means ± SEM. N = 8 in all groups studied. Statistical significances according to Student's t-test.

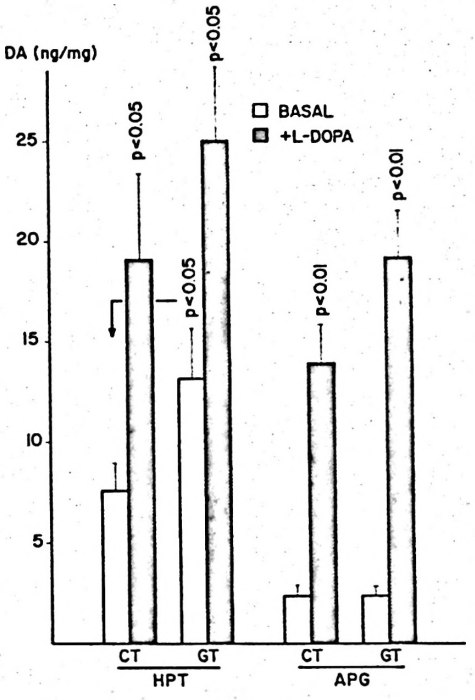

Fig. 2. *Hypothalamic and* in situ *anterior pituitary dopamine (DA) contents, both basally and 90 min after L-dopa administration (50 mg/kg weight), in grafted (GT) and control (CT) animals.*
Legend as in figure 1.

tration in both grafted and control animals. No differences could be detected in pituitary DA contents between both groups, but the increase observed in grafted rats after L-dopa administration was greater than the observed in control animals.

Figure 3 shows, hypothalamic and anterior pituitary NE contents after L-dopa or saline administration to both control and grafted rats. One month after the transplant operation, grafted rats exhibited an increase in the hypothalamic NE content as compared to controls. Administration of L-dopa led to an increase in hypothalamic NE content of controls but no differences were shown in grafted animals. Norepinephrine was not detectable in the anterior pituitary, nor basally, nor after L-dopa administration, in both grafted and control animals.

Discussion

The grafting of one anterior pituitary gland under the kidney capsule resulted in a very well known increase in plasma PRL levels (4, 36). Besides these modifications a decrease in plasma GH levels can be detected. This effect has been only reported in women with hyperprolactinemia of different ethiologies (2, 19).

Various data from the literature have shown that PRL is able to modulate other pituitary hormone secretions through its action at the hypothalamic level, mainly modifying DA turnover (3, 6, 10, 13), which allows to suspect that this hormone may exert its regulatory effects on GH by modifying the activity of several hypo-

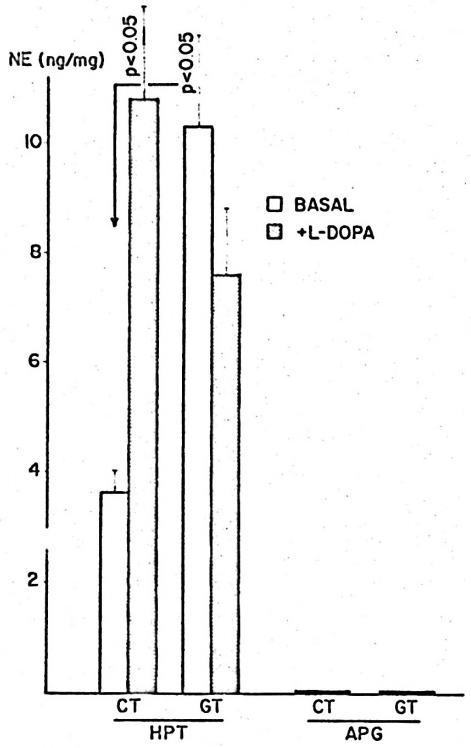

Fig. 3. *Hypothalamic and* in situ *anterior pituitary norepinephrine (NE) contents, both basally and 90 min after L-dopa administration (50 mg/kg weight), in grafted (GT) and control (CT) animals.* Legend as in figure 1.

thalamic neurotransmitters. This study clearly shows that the increase in hypothalamic DA activity observed after the grafting would exert an inhibitory effect on GH secretion in grafted animals. This was confirmed after L-dopa administration. Hypothalamic NE activity was also increased after the grafting, however L-dopa administration led to an increase in controls but not in grafted animals, which suggests a most important role for DA in the PRL induced changes on GH secretion. Possible PRL induced modifications in other neurotransmitters that may act on GH secretion cannot be excluded (22, 24).

These facts support the existence of a hypothalamic site of action for these neurotransmitters in the control of GH secretion, probably modifying the release of GH-releasing factor (GHRF) and/or somatostatin to the portal blood. This was previously proposed by some authors (9, 26, 28, 32, 38). At this respect, recent data have shown the coexistence of tyrosine hydroxylase activity and GHRF in medio basal hypothalamic neurons (31, 33), suggesting that DA may exert a modulatory effect on the GHRF secretion.

However, the possibility that one pituitary cell type could be responsible of both GH and PRL secretions, as has been repeatedly suggested (5, 15, 18), could also support the decrease of GH secretion after L-dopa administration, through a possible direct action of DA on these cells. At this respect, the marked increase in anterior pituitary DA content observed after L-dopa administration could be responsible not only of the decreased PRL secretion (14, 16), but also of the decrease in GH release.

In conclusion, these data suggest that PRL could be able to modify GH secretion. This effect seems to be mainly exerted through changes in the DA action on GH secretion at hypothalamic and/or anterior pituitary level.

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Resumen

Se estudiada la existencia de mecanismos de interrelación entre prolactina (PRL) y hormona de crecimiento (GH). El estudio se ha realizado en ratas macho adultas portadoras de un implante adenohipofisario bajo la cápsula renal desde el día 90 de vida y en sus correspondientes controles con operación

simulada. Los animales fueron inyectados con una única dosis de L-dopa (50 mg/kg de peso) o solución salina a los 30 días de la operación de implante. Los niveles plasmáticos de PRL y GH se midieron mediante radioinmunoanálisis específicos. Los contenidos de dopamina (DA) y norepinefrina (NE) en el hipotálamo y en la adenohipófisis *in situ* se midieron mediante un método radioenzimático específico. Los niveles plasmáticos de PRL se incrementaron, mientras que los niveles de GH disminuyeron, en los animales con implante adenohipofisario. Los contenidos hipotalámicos de DA y NE se incrementaron en estos animales, mientras que el contenido adenohipofisario de DA no se modificó respecto de los controles. La administración de una única inyección de L-dopa redujo los niveles plasmáticos de PRL y de GH, tanto en animales con implante como en animales controles, pero mientras se observó un notable incremento en los contenidos hipotalámicos y adenohipofisarios de DA en ambos grupos, el contenido hipotalámico de NE sólo se incrementó en animales controles. Estos datos sugieren que la secreción de PRL y la de GH están estrechamente relacionadas. Dopamina podría ejercer una acción mediadora en la acción de PRL sobre GH, mientras que NE estaría menos implicada.

Palabras clave: Prolactina, Hormona de crecimiento, Dopamina, Norepinefrina.

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