PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1128–1131 (1974)

# Reciprocal Relationship Between the *in Vitro* Synthesis and Secretion of Prolactin and Growth Hormone[1] (37966)

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Studies in this laboratory have been primarily concerned with the control mechanisms governing the production of pituitary gland hormones. We have been particularly interested in the production of the two closely related hormones, prolactin and growth hormone (1–3). Since the primary structures of these two hormones are somewhat similar, each has some biological activity of the other (4). There is sufficient difference between them, however, such that each has very distinct and unique biological functions. It has been shown that the neuroendocrine and pharmacological control of the production of each of these hormones is primarily regulated by very different mechanisms (5). Nevertheless, during the course of our experimentation we have observed that a reciprocal relationship exists between the production of the two hormones. In the present study we have attempted to explore this relationship.

*Materials and Methods.* Mature male and female rats of the Sprague–Dawley strain were obtained from Flow Research Animals, Dublin, VA. The rats were routinely housed 4–5 to a cage and allowed water and Purina Laboratory Chow *ad lib.* Pituitary tumor 7315a was transplanted into mature female rats of the Buffalo strain (obtained from Simonsen Laboratories, Gilroy, CA) as previously described (1).

Following decapitation of the animals, the anterior pituitary glands were quickly excised and hemisected. The glands were divided such that each incubation flask contained 4 hemipituitary glands in 1 ml of tissue culture Medium 199 (Microbiological Associates, Inc., Bethesda, MD) and 10 $\mu$Ci 4,5-$^3$H-leucine (International Chemical and Nuclear, Irvine, CA, 50 Ci/mmole). The flasks were incubated in a Dubnoff shaker at 37° for 6–7 hr in an atmosphere of 95% $O_2$–5% $CO_2$. At the termination of the incubation, the pituitary glands were homogenized in 1 ml of 0.05 *M* phosphate, pH 7.2, using a glass homogenizer fitted with a Teflon pestle (Kontes Glass Co.). The homogenates were frozen and thawed three times to lyse the granules. Duplicate 25- or 50-$\mu$l aliquots of the homogenates and the incubation media were subjected to polyacrylamide gel electrophoresis according to the Reisfeld *et al.* (6) modification of the method of Jones *et al.* (7). The protein bands on the gels were stained with amido black and identified by comparing their electrophoretic patterns with those produced by purified rat prolactin and growth hormone. The authenticity of the prolactin and growth hormone bands on polyacrylamide gels has been documented by many investigators. The protein bands were dissolved in 30% $H_2O_2$ at 80° and the radioactivity determined by liquid scintillation techniques.

Perphenazine was a gift from Schering Corp. Haloperidol was obtained from McNeil Laboratories. Polyestradiol phos-

[1] A preliminary report of this work was presented at the IV International Congress of Endocrinology, Washington, D. C., 1972. Supported by a grant from the National Cancer Institute, NIH, CA-07535.

[2] Research Career Awardee CA-07665 during the initial portion of this work.

phate (Estradurin) was a gift from the Ayerst Laboratories, Inc.

In most instances each group was composed of three flasks and each figure represents a representative experiment of replicates.

Student's *t* test was used for the statistical evaluation of the data and the results are expressed as the mean ± SEM.

*Results.* Anterior pituitary glands of female and male rats were incubated for 6–7 hr in the presence of $^3$H-leucine. The incorporation of the labeled amino acid into prolactin and growth hormone is shown in Fig. 1. In this figure, the open portion of the bar indicates the amount of newly synthesized hormone found in the incubation medium at the termination of the incubation, while the shaded portion represents the labeled hormone found within the tissue. When placed *in vitro,* the normal pituitary gland of the female synthesizes and releases a large amount of prolactin, while the gland of the male produces much less ($P < 0.01$). In this particular experiment, the glands of male animals synthesized only an eighth as much prolactin as did the glands of females. The situation is reversed with growth hormone; the glands of the males synthesized more than twice as much growth hormone as the glands of the females ($P < 0.01$). While prolactin is rapidly released into the medium, growth hormone tends to be retained within the tissue.

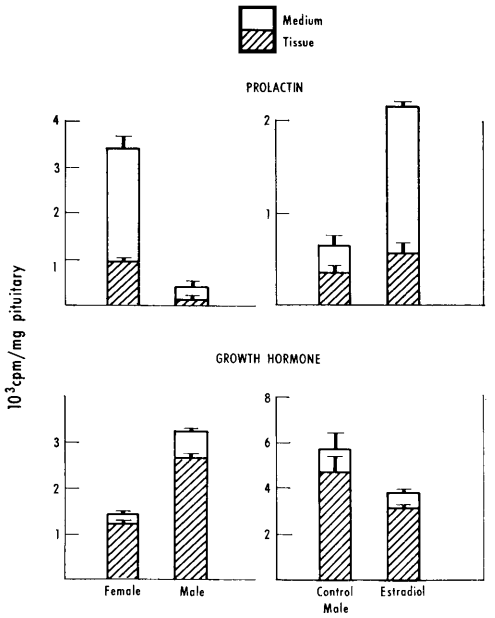


FIG. 1. (Left) The *in vitro* incorporation of 4,5-$^3$H-leucine into pituitary gland hormones of male and female rats; $P < 0.01$. (Right) The effect of 100 μg polyestradiol phosphate administered 7 days before sacrifice to male rats on the incorporation of labeled amino acid into prolactin and growth hormone; $P < 0.01$. In all figures the open portion of the bar indicates the amount of labeled hormone secreted into the incubation medium while the shaded portion indicates the amount retained in the pituitary gland.

The ability of estradiol to enhance prolactin production is well-established. Figure 1 also illustrates the effect of the administration of estradiol on the *in vitro* function of pituitary glands of male rats. As a result of administration of the steroid, *in vitro* prolactin synthesis was increased almost three-fold. At the same time, growth hormone production was significantly reduced.

Recent studies have indicated that brain catecholamines inhibit the release of prolactin by the pituitary gland. Agents that deplete the brain catecholamine stores, or block the catecholamine receptor sites, are potent stimuli of prolactin production (8, 9). When male rats were given 3 or 4 daily injections of 1 mg perphenazine, the *in vitro* prolactin synthesis was greatly enhanced (Fig. 2, left side). The action on growth hormone, however, was precisely the opposite. Growth hormone synthesis was profoundly decreased. Treatment of male rats, with 4 daily injections of 0.1 mg haloperidol gave almost identical results (Fig. 2, right side). *In vitro* prolactin synthesis and release were greatly stimulated while growth hormone production was reduced.

The pituitary glands of animals bearing hormone-secreting pituitary tumors are atrophic and contain decreased amounts of prolactin or growth hormone (1). Pituitary tumor 7315a secretes large amounts of prolactin which inhibit prolactin production by the pituitary gland of the host animal. The *in vitro* production of prolactin and growth hormone by these glands is shown

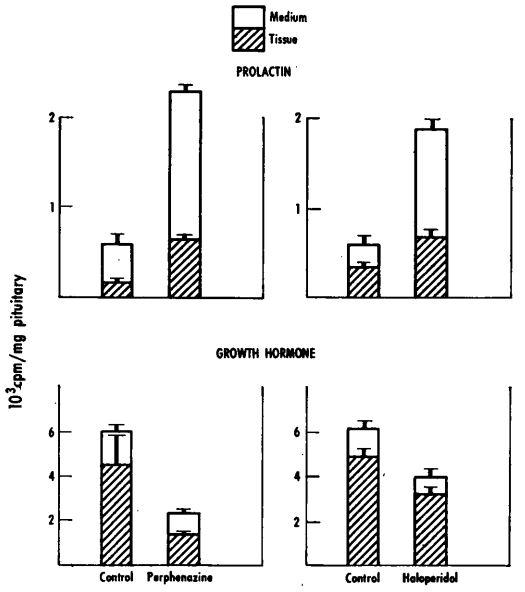


FIG. 2. (Left) The *in vitro* incorporation of 4,5-$^3$H-leucine into prolactin and growth hormone by pituitary glands of male rats injected with perphenazine or haloperidol (right side). $P < 0.01$.

in Fig. 3. The pituitary glands of rats implanted with this particular tumor synthesized only about 40% as much prolactin as did their normal controls. On the other hand, they synthesized 76% more growth hormone.

The effect of dibutyryl cyclic AMP on pituitary hormone production is of particular interest. As shown in Fig. 4, the addition of $5 \times 10^{-3}$ *M* dibutyryl cyclic AMP to the incubation medium caused an increase in the amount of newly synthesized

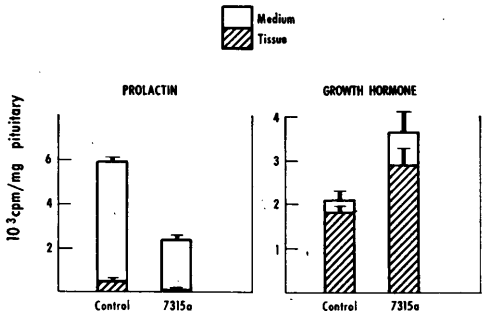


FIG. 3. The *in vitro* incorporation of labeled leucine into pituitary gland hormones of female rats bearing the pituitary tumor 7315a. $P < 0.01$.

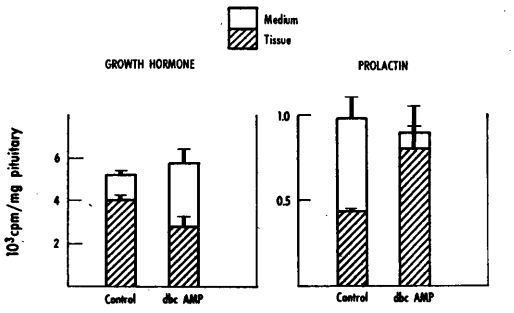


FIG. 4. The effect of $3 \times 10^{-3}$ *M* dibutyryl cyclic AMP (dbc AMP) on the *in vitro* secretion of radioactive growth hormone and prolactin by the pituitary glands of male rats. $P < 0.01$.

growth hormone released into the medium (10). There was a concomitant decrease in the amount of newly synthesized growth hormone contained within the pituitary gland. Consequently, there was no change in growth hormone synthesis. With respect to prolactin, the nucleotide caused a decrease in the amount of prolactin released into the medium and an increase in the amount retained within the tissue. Again, total hormone synthesis was not changed.

*Discussion.* The data presented here demonstrate the existence of an incidental reciprocal relationship between the production of prolactin and that of growth hormone. Ben-David *et al.* (11) previously presented evidence that antagonism exists between the secretion of prolactin and gonadotrophin. We have consistently observed that when the *in vitro* production of prolactin is stimulated there is a concomitant fall in growth hormone synthesis. This condition, however, occurs naturally: the pituitary glands of female rats synthesize more prolactin than growth hormone while the reverse is true of glands of male rats. The present studies show that this situation can be induced by pharmacological manipulation. There may be multiple explanations for this phenomenon but the two which may have greatest merit are: (a) it is possible that these drugs have a stimulatory effect on one system and an inhibitory effect on the other or (b) it may be that the increase in prolactin production has a direct inhibitory effect on the production of growth

hormone and vice versa. It would appear that the sum of prolactin and growth hormone production proceeds at a fixed rate and any environmental influence which increases the production of one hormone causes a compensatory decrease in the production of the other so that a constant rate of total hormone production is maintained. The experiments with dibutyryl cyclic AMP demonstrate that an agent can simultaneously cause opposite changes in release of prolactin and growth hormone without altering the synthesis of the hormones. The increase or decrease in release is accompanied by a respective decrease or increase in the amount of hormone accumulated within the tissue such that no overall change in hormone production occurs.

Although earlier experiments were unable to establish any consistent effect of dibutyryl cyclic AMP on the *in vitro* release of newly synthesized prolactin by pituitary glands from male rats, more recent data have been more uniform in demonstrating that the nucleotide does release the *in vitro* secretion of prolactin.

*Summary.* A comparison was made of the amount of radioactive leucine incorporated *in vitro* into prolactin and growth hormone by rat pituitary glands. It was demonstrated that when the production of prolactin is high, the production of growth hormone is low. The converse of this was also observed. The injection of male rats with estradiol or other agents which significantly increased prolactin production caused a decrease in growth hormone production. It is suggested that the pituitary gland tends to produce a constant amount of prolactin and growth hormone and reciprocal changes are brought about by the production of either hormone through some intracellular control mechanism when the hormonal balance is disturbed through a secondary mechanism.

We thank Mr. Ronald C. Pace for his excellent technical assistance.

1. MacLeod, R. M., Smith, M. C., and DeWitt, G. W., Endocrinology **79**, 1149 (1966).

2. MacLeod, R. M., and Abad, A., Endocrinology **83**, 799 (1968).

3. MacLeod, R. M., Abad, A., and Eidson, L. L., Endocrinology **84**, 1475 (1969).

4. Li, C. H., *in* "Growth and Growth Hormone" (A. Pecile and E. E. Muller, eds.), p. 17, Excerpta Medica, Amsterdam (1972).

5. Geschwind, I. I., *in* "Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry" (J. Meites, ed.), p. 298, Williams and Wilkins, Baltimore (1970).

6. Reisfeld, R. A., Lewis, U. J., and Williams, D. E., Nature **195**, 281 (1962).

7. Jones, A. E., Fisher, J. N., Lewis, U. J., and Vanderlaan, W. P., Endocrinology **76**, 578 (1965).

8. MacLeod, R. M., Fontham, E. H., and Lehmeyer, J. E., Neuroendocrinology **6**, 283 (1970).

9. Ben-David, M., Danon, A., Benveniste, R., Weller, C. P., and Sulman, F. G., J. Endocrinol. **50**, 599 (1971).

10. MacLeod, R. M., and Lehmeyer, J. E., Proc. Nat. Acad. Sci. USA **67**, 1172 (1970).

11. Ben-David, M., Danon, A., and Sulman, F. G., J. Endocrinol. **5**, 719 (1971).

Received Sept. 4, 1973. P.S.E.B.M., 1974, Vol. 145.