Cell proliferation in the rat pituitary gland: A mechanism of control in prolactin cells[1]

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Summary. We investigated the relationship between prolactin content and DNA replication in the anterior pituitary gland. Thymidine incorporation in pregnant rats is significantly lower than in virgin controls. This is accompanied by a decreased activity of DNA polymerase. Sulpiride administration to pregnant rats enhances thymidine incorporation to levels similar to virgin controls. The results indicate a negative feedback between prolactin content and DNA synthesis in the rat anterior pituitary gland.

Cell proliferation in the anterior pituitary gland (APG) changes with the physiological states of the animal[2]. Certain hormones, like oestrogens, stimulate DNA synthesis in acidophilic mammotrophs and can also induce tumors in this gland[3,4], whereas castration stimulates DNA replication in basophilic gonadotrophs[5]. Mitosis increases in the gland when TSH release is stimulated with TRH[6] and when ACTH formation increases after adrenalectomy[7]. On the other hand, bromocryptine is a drug that inhibits release of prolactin and also DNA synthesis in pituitary gland stimulated by oestrogens[8]. We investigated if the intracellular prolactin content is a mechanism of control of cell proliferation in the APG by measuring thymidine incorporation into DNA, as well as the activities of 3 enzymes related to DNA synthesis (DNA polymerase, thymidine kinase and an endonuclease which has the capacity of activating the DNA template for the DNA polymerase assay[9]). For this purpose we used rats in their last week of pregnancy, a well-established condition when the gland is almost entirely composed of mammotrophs with a very high prolactin content[10]. We also employed certain drugs with a well known, positive or negative, effect on the release of prolactin: sulpiride[11] and bromocryptine, respectively.

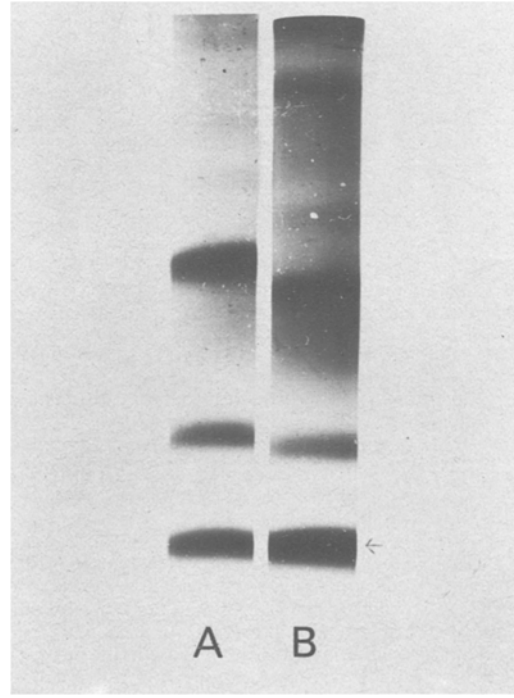
Our results indicate the existence of a negative feedback between the intracellular concentration of prolactin and DNA replication in the APG, and also that an acute release of the hormone can trigger DNA synthesis. There is also a significant decrease in the activity of DNA polymerase in pregnant rats with respect to virgin controls.

Material and methods. Female Wistar rats (3–4 months old) were maintained in a light and temperature controlled room on tap water and food ad-libitum. Control animals were virgins, and pregnant rats were between 1 and 4 days before delivery. Sulpiride sulfate (5 mg/rat in 0.5 ml of 0.14 M NaCl) and bromocryptine (2 mg/rat in 0.5 ml of 70% ethanol: 0.14 M NaCl, 1:1.5, v/v) were injected s.c. 20 h before decapitation of the animals, and controls received the same volume of the vehicle. The pituitary gland was removed, the posterior lobe discarded, the APG halved and transferred to a chilled tube containing 0.5 ml of medium TC199 and incubated under 95% O_2–5% CO_2 at 37 °C in a metabolic shaker. After 10 min, 2 µCi of (Me-^{3}H) thymidine (specific radioactivity 50.8 Ci/mmole) were added and the incubation continued for another 60 min. During this time the incorporation of the precursor was linear. At the end of incubation, the medium was rapidly removed and the APGs were washed twice with 1 ml of cold 0.14 M NaCl. The tissue was then homogenized in 1 ml 10% (w/v) cold trichloroacetic acid solution (TCA) and centrifuged at 6500× g for 15 min.

The insoluble residue was washed twice with 1 ml of 5% (w/v) TCA solution and dissolved in 0.3 ml of 0.5 N NaOH. Aliquots of this solution were taken to determine: a) DNA by the diphenylamine reaction with native DNA as a standard[12], and b) radioactivity in vials containing 10 ml of Bray's solution and 500 mg of Cab-O-Sil M_5 (Cabot, Argentina). The supernatants were used to measure the radioactivity in the soluble fractions and represents the precursor uptake by the tissue. Counts were corrected to 100% efficiency by the channel ratio method.

To assay DNA polymerase, endonuclease and thymidine kinase, 2 APGs were homogenized in 1 ml 10 mM Tris-HCl (pH 7.4), 2 mM β-mercaptoethanol and centrifuged 20 min at 24150× g. The supernatants were used to determine enzymatic activities and protein content according to methods previously described[13,14]. Serum prolactin was measured by radioinmunoassay 30 min after the injection of sulpiride sulfate[15].

Results and discussion. The APG in pregnant rats contains considerably more prolactin than the virgin controls and this can be seen by polyacrilamide gel electrophoresis (figure). This is accompanied by a decreased incorporation of tritiated thymidine into DNA, indicative of reduced cell proliferation (table 1). The administration of sulpiride to pregnant rats increases DNA replication to levels similar to the nonpregnant animals (table 1). This drug is known to produce liberation of prolactin from the APG into the blood stream (table 2) which results in a transitory hormone depletion of the gland. When bromocryptine is administered together with sulpiride, the stimulation in cell



Disc gel electrophoresis of pituitary gland from virgin and pregnant rats. APG extracts were prepared according to Zanini and Giannattasio's[18]. Polyacrilamide gel electrophoresis was performed according to Davies[19] with 200 µg of protein per gel. *A* virgin control; *B* pregnant rat. The arrow indicates the prolactin band.

proliferation by sulpiride is markedly reduced, although it does not reach the levels of the control pregnant rats (table 1). The bromocryptine acts by inhibiting prolactin release. The administration of bromocryptine alone has no effect on DNA synthesis in the APG of pregnant rats (table 1). The activity of endonuclease and thymidine kinase is similar in pregnant and virgin rats, while that of soluble DNA polymerase diminishes in pregnant animals (table 3).

Our results indicate that the intracellular prolactin content is an important factor in the regulation of DNA replication in the APG. Clearly this is independent of the serum prolactin levels, because during late pregnancy there is a decreased DNA synthesis accompanied by hyperprolactinemia, while sulpiride administration to pregnant rats stimulates DNA replication by an acute prolactin release from the APG that increases the prolactinemia even more.

Although we have not seen changes in the prolactin content by disc gel electrophoresis 20 h after sulpiride treatment, it is possible that an acute release of prolactin from the APG is followed by an increased synthesis of the hormone which rapidly restores the prolactin content of the gland. Mechanisms of this kind have been described in the APG[16]. This would mean that an acute transitory depletion of prolactin is enough to trigger a series of events which results in an increased DNA synthesis in the APG. A similar effect was observed in pancreatic beta cells after release of insulin evoked by adding D-glucose to the culture medium[17]. An alternative explanation for our results is a direct action of the drugs employed on DNA replication. However, this seems unlikely, because nontreated pregnant rats also have a considerably lower DNA synthesis than the virgin controls. Also, bromocryptine partially reverts the effect of sulpiride on DNA synthesis. The 2 drugs have specific antagonistic actions on the release of prolactin from the APG.

The lack of response to bromocryptine alone in the pregnant rats might indicate that the mechanism of action of this drug depends on the elevation of the intracellular content of prolactin in the APG, which during pregnancy could be already high enough to produce maximal inhibition of DNA synthesis. Among the enzymatic activities studied, only the soluble DNA polymerase decreases in pregnant rats. Endonuclease and thymidine kinase activities remain within control values. The mechanism by which the intracellular content of prolactin regulates DNA synthesis in the APG remains to be investigated.

Although a mechanism that controls cell proliferation in the APG seems to depend on the intracellular content of prolactin, prolactinomas have a high content of this hormone and they still continue to grow. It might be that tumours of the APG have lost this intracellular feedback mechanism.

Table 1. In vitro incorporation of tritiated thymidine into DNA of rat pituitary gland: Effect of pregnancy and of sulpiride sulfate and bromocryptine treatment on pregnant rats

Animals	Treatment	Average of relative specific radioactivities ± SE
1. Virgin controls	Vehicle	142 ± 28
2. Pregnant	Vehicle	52 ± 5**
3. Pregnant	Sulpiride	166 ± 18***
4. Pregnant	Sulpiride + bromocryptine	88 / 72*
5. Pregnant	Bromocryptine	67 / 39*

$$\text{Relative specific radioactivity is} = \frac{\text{insoluble dpm/mg DNA}}{\text{soluble dpm/mg DNA}} \times 1000$$

dpm/mg DNA in the TCA insoluble residue was 90.708 ± 9.847 in virgin controls. The mean value of the dpm/mg DNA in the TCA soluble fraction of all the experimental groups was 608.148 ± 53.291. Each value represents the mean ± SE of 6 determinations. *Individual results of the 2 different experiments. **$p < 0.005$ by Student's 't'-test (pregnant vs. virgin control). ***$p < 0.002$ by Student's 't'-test (pregnant vs. pregnant treated with sulpiride).

Table 2. Serum prolactin levels in pregnant rats: Effect of sulpiride

Treatment	ng of prolactin/ml serum
Vehicle	300 ± 100
Sulpiride	> of 4000

The results are the average of 4 determinations ± SE. Serum samples were obtained 30 min after sulpiride injection.

Table 3. Effect of pregnancy on the activity of DNA polymerase, thymidine kinase and endonuclease in the rat pituitary gland

Animal	pmoles TMP incorporated/ mg protein/ 30 min ± SE DNA polymerase	pmoles TMP formed/ mg protein/ 10 min ± SE Thymidine kinase	% stimulation/ 100 µg of protein ± SE Endonuclease
Control	12.9 ± 1.0	50.1 ± 5.2	417 ± 86
	$p < 0.005$*	NS*	NS*
Pregnant	10.6 ± 1.0	54.0 ± 7.1	410 ± 55

For the DNA polymerase and thymidine kinase reaction, 150–200 µg of protein per tube were used. For the endonuclease assay, the native DNA was preincubated for 90 min at 37 °C with 40 µg of protein and then heat inactivated at 60 °C for 10 min. The percentage stimulation was determined as an increase in the incorporation of ^{3}HTTP into a TCA insoluble product using this DNA as a primer in comparison with native DNA. The DNA polymerase for the assay was partially purified from rat brain (fraction IIa + IIb)[12]. The results are the average of 8 experiments for the DNA polymerase and endonuclease assays and 4 experiments for the thymidine kinase assay ± SE. *By Student's paired 't'-test. NS, not significant.

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