DNA synthesis in the pituitary gland of the rat: Effect of sulpiride and clomiphene[1]

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Summary. Sulpiride administration to rats releases prolactin and increases DNA replication in the anterior pituitary gland. Clomiphene prevents the stimulation of DNA synthesis produced by sulpiride, but does not affect prolactin release from the gland. These findings suggest that the intracellular prolactin content of the anterior pituitary gland plays a role in the regulation of DNA synthesis through a mechanism mediated by oestrogens.

During pregnancy, when the pituitary prolactin content is higher than in virgin controls, DNA synthesis in the APG[2] is depressed[3]. Sulpiride is a drug that stimulates prolactin release[4] and also DNA synthesis in the APG[3], while bromoergocryptine inhibits the prolactin release[5] and partially prevents the effects of sulpiride[3] and also the stimulatory effect on pituitary cell proliferation produced by oestrogens[5].

This evidence, so far, indicates that in the pituitary gland mammotrophs the intracellular prolactin content is important in the regulation of DNA synthesis. On the other hand, it is well established that oestrogens play a role in DNA replication in prolactin cells[6].

Our purpose was to study the relationship between the effect of sulpiride that produces prolactin release from the APG and increases DNA replication, and clomiphene that blocks oestrogen receptors[7].

Materials and methods. Rats from a highly inbred Wistar strain were used. Pregnant animals were between 1 and 4 days before delivery and males were between 180 and 220 g. Sulpiride sulphate (5 mg/rat in 0.5 ml of 0.14 M NaCl) was injected s.c. 20 h before decapitation of the animal. Clomiphene citrate was prepared as a 2% suspension in carboxymethyl cellulose 1% in water and 2 mg/rat was injected s.c. 48 and 20 h before decapitation of the animal. Control rats received the same volume of the vehicle. The pituitary glands were removed, the posterior lobe discarded, the APGs halved and transferred to chilled tubes containing 0.5 ml of medium TC 199 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) was 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 removed, and the APGs washed twice with 1 ml of cold 0.14 M NaCl. The tissue was then homogenized in 1 ml 10% (w/v) cold TCA[2] and centrifuged at 6500×g for 15 min. The insoluble residue was washed twice with 1 ml 5% (w/v) TCA solution and dissolved in 0.9 ml of 0.5N NaOH. Aliquots of this solution were taken to determine: a) DNA by the diphenylamine reaction with native DNA as a standard[8] 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 fraction and represent the precursor uptake by the tissue. Counts are corrected to 100% efficiency by the Channel ratio method. Serum prolactin was measured 30 min after the injection of sulpiride sulfate by radioimmunoassay[9].

Results. The administration of sulpiride to pregnant rats increases the incorporation of tritiated thymidine 3 to 4 times. On the other hand, if the pregnant rats are pretreated with clomiphene, the stimulatory effect of sulpiride is completely abolished. Clomiphene itself diminishes the incorporation of the precursor into DNA in the APG (table 1).

Sulpiride produces an acute prolactin release from the APG as can be seen by the increased serum prolactin levels 30 min after the administration of the drug. Although the enhanced thymidine incorporation produced by sulpiride is abolished by clomiphene, the prolactin release still occured. Clomiphene injected alone into pregnant rats does not change the prolactinemia (table 2). In male rats sulpiride produces the same effect on DNA replication but the percentage of change is quantitatively lower than in pregnant rats. This is probably due to the different population

Table 1. In vitro incorporation of tritiated thymidine into DNA of pregnant rat pituitary gland: Effect of sulpiride and clomiphene

Treatment	Average of relative specific radioactivities ± SE	Change (%)
Vehicle	87 ± 6	
Sulpiride	360 ± 39*	+314
Sulpiride + clomiphene	87 ± 11	0
Clomiphene	40 ± 5**	− 54

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

dpm/mg DNA in the TCA insoluble residue was 210,086 ± 23,280 in pregnant untreated rats. The mean value of dpm/mg DNA in the TCA soluble fraction of all experimental group was: 2,426,280 ± 272,700. Each value represents the mean ± SE of 5 determinations. * $p < 0.001$ by Student's t-test. ** $p < 0.005$ by Student's t-test.

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

Treatment	ng of prolactin/ml serum
Vehicle	300 ± 100
Sulpiride	> of 4000
Sulpiride + clomiphene	> of 4000
Clomiphene	140 ± 48

The results are the average of 4 determinations ± SE. Serum samples were obtained 30 min after sulpiride injection. Prolactin was measured by radioimmunoassay using the double antibody method according to Niswender et al.[9].

Table 3. In vitro incorporation of tritiated thymidine into DNA of male rat pituitary gland: Effect of sulpiride and clomiphene

Treatment	Average of relative specific radioactivities ± SE	Change (%)
Vehicle	144 ± 18	
Sulpiride	242 ± 31*	+68
Sulpiride + clomiphene	125 ± 11	−13
Clomiphene	120 ± 12	−16

dpm/mg DNA in the TCA insoluble residue was 279,875 ± 29,810 in male untreated rats. The mean value of dpm/mg DNA in the TCA soluble fraction in all experimental groups was: 1,943,583 ± 208,172. Each value represents the mean ± SE of 5 determinations. * $p < 0.01$ by Student's t-test.

of pituitary prolactin cells in male and pregnant rats. The APG of pregnant rats is primarily composed of mammotrophs. In male rats clomiphene also abolishes the effect of sulpiride on the incorporation of tritiated thymidine in the APG. In this respect the results are similar to those obtained with pregnant animals (table 3).

Discussion. The results of these experiments confirm our previous work on the relationship between prolactin content and DNA synthesis in the APG. In APGs with a high intracellular prolactin content (as in pregnant rats) DNA replication is markedly depressed, but when prolactin is released with sulpiride, DNA synthesis increases. This last effect is abolished by clomiphene, which is an oestrogen receptor blocking agent. This suggest that the intracellular prolactin content in the APG plays a role in the regulation of DNA synthesis through a mechanism mediated by oestrogens. It appears that prolactin depletion from the APG enhances the oestrogenic action on prolaction cells resulting in a stimulation of cell proliferation. On the other hand, clomiphene may alter the release of hypothalamic hormones and of pituitary gonadotropins and as a result of this alteration may block the stimulation of DNA synthesis produced by sulpiride.

A similar effect is observed in male rats. There is strong evidence that androgens are aromatized to oestrogens in the APG[10], and that this may be the mechanism by which the androgenic hormones affect prolactin cells. When several androgenic steroids were administered to male rats, serum prolactin increased only with those which could be aromatized to oestrogens[11]. Therefore we propose that, in male and female rats, prolactin cell proliferation could be regulated by a similar mechanism.

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2 Abbreviations: APG, anterior pituitary gland; TCA, trichloroacetic acid.

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Substance P in medullary carcinoma of the thyroid

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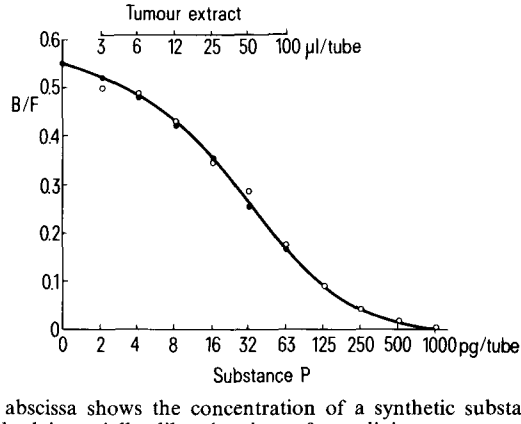
Summary. High levels of substance P-like immunoreactivity were demonstrated by radioimmunoassay in the plasma and tumour of a patient with a medullary carcinoma of the thyroid.

Medullary carcinoma of the thyroid (MCT) is an endocrine tumour capable of producing calcitonin, somatostatin, ACTH, histaminase, prostaglandins, and serotonin (5-HT)[1-3]. Light and electron-microscopic studies show a close resemblance between MCT and carcinoid tumours and several reports have described the carcinoid syndrome in patients with MCT.

We have reported previously on the production of substance P (SP) by carcinoid tumours[4,5]. We now report the finding of immunoreactive SP in the plasma and tumour of a patient with MCT.

Materials and methods. Tumour tissue was removed at autopsy from a 65-year-old man with a mediastinal mass and the carcinoid syndrome. The tumour was found in the left thyroid lobe extending into the anterior mediastinum and metastasizing into thoracic lymphnodes and liver. Histology of the tumour and metastases showed a typical MCT with abundant amyloid and argentophilia. The tumour and adjacent normal thyroid tissue were extracted according to the method of Chang and Leeman[6], the extract was evaporated and the residues dissolved in assay buffer, pH 7.6, and assayed for calcitonin and SP[8,9]. Blood was withdrawn into 10-ml plastic tubes containing 150 I.U. of heparin, spun, and the plasma was assayed for calcitonin and SP. The antiserum used in the SP radioimmunoassay was characterized previously and shown to be directed against the biologically active end of the SP molecule, i.e. its C-terminus[10]. The sensitivity of the assay is 2 pg, i.e. 1.5 fmoles[9]. The urinary 5-hydroxyindole-acetic acid was 15 mg/24 h.

Results and discussion. The tumour content of calcitonin was 1.25 µg/g wet tissue. The plasma level of calcitonin was 50 ng/ml. The tumour content of SP was 48 ng/g wet tissue. Serial dilution of the tumour extract showed a close parallelism with the SP standard (MRC, London) and is shown in the figure. The normal thyroid gland contained less than



The abscissa shows the concentration of a synthetic substance P standard in serially diluted points of a radioimmunoassay curve (open circles). The ordinate shows the antibody binding as the bound over free ratio corrected for 'damage' of the tracer. The closed circles superimposed on the standard curve represent values of antibody displacement for the tumour extract. The volume of the extract reconstituted in the assay buffer is shown on the upper scale.