Pergamon

0960-0760(94)00121-9

J. Steroid Biochem. Molec. Biol. Vol. 51, No. 3/4, pp. 183–189, 1994

0960-0760/94 $7.00 + 0.00

Biochemical Parameters in the Anterior Pituitary During the Course of Tumorigenesis Induced by Diethylstilbestrol Treatment

Gerardo Piroli, Analia E. Lima, Graciela Díaz-Torga and Alejandro F. De Nicola*

Laboratorio de Bioquímica Neuroendócrina, Instituto de Biología y Medicina Experimental-CONICET and Química Biomédica, Facultad de Medicina, U.B.A., Buenos Aires, Argentina

Treatment of F_{344} rats with diethylstilbestrol (DES) for 1–2 months induces a prolactin (PRL)-secreting pituitary adenoma. After 8 weeks of DES treatment, we have shown that the ratio of regulatory subunits of the cAMP-dependent protein kinase (RI/RII) increased in the tumors. Presently we report the variations in RI/RII ratio, pituitary weight, DNA content, serum PRL, nuclear estrogen receptor (E_2R) and of ornithine decarboxylase (ODC) activity from the time of DES pellet implantation until 8 weeks. Pituitary weight, DNA content and serum PRL rose significantly at 4 weeks with a maximum at 6–8 weeks, and significantly correlated with each other. E_2R and ODC activity increased from week 1 onwards, with a maximum at 2 weeks and decreased at 8 weeks. Both variables showed a positive correlation but neither E_2R nor ODC activity correlated with pituitary weight, DNA or serum PRL. Values for RI remained stable with time, but RII decreased progressively. The RI/RII ratio was maintained around unity between 1–4 weeks, increasing to 1.6–2 thereafter. This ratio positively correlated with pituitary weight and DNA. It is suggested that during tumor induction by estrogen in a sensitive strain of rats, growth signals with different time-courses become activated. Increases in pituitary weight and DNA content, indicators of mammotroph hypertrophy and hyperplasia, were preceeded by early rises in E_2R and ODC activity. Increases in the RI/RII ratio accompanied the adenomatous change, suggesting their role in cell transformation after 6 weeks of estrogen exposure.

J. Steroid Biochem. Molec. Biol., Vol. 51, No. 3/4, pp. 183–189, 1994

INTRODUCTION

Chronic treatment with estrogens induces a prolactin (PRL)-secreting pituitary adenoma [1]. Tumor induction is facilitated in Fischer 344 (F_{344}) rats which are genetically hypersensitive to estrogens [2]. Histologically the tumors are composed of hyperplastic and hypertrophied mammotrophs, with involution of somatotrophs and gonadotrophin-producing cells [3, 4].

Emphasis has been placed on the hypothalamus as the anatomical site of action for estrogen induction of pituitary tumors, due to the suppression of tuberoinfundibular dopaminergic neurons by estrogen or high PRL titers [5–7]. However, the presence of very high affinity estrogen receptors (E_2R) in the adenomata, supports a direct hormonal effect at the pituitary level [8, 9]. The high rate of DNA synthesis [4] and of DNA polymerase α activity in the tumors [10] as well as the report of an estrogen-responsive element in the 5′-flanking region of the PRL gen in the tumors [11] are consistent with direct stimulation of mammotroph proliferation by estrogens [12]. However, the messengers involved in this proliferative effect are not clearly defined. In another model of hormone-dependent malignancy, the interaction between E_2R and cyclic adenosine monophospate (cAMP) binding proteins has been postulated to modify gen expression [13]. In this respect, cAMP binding to the regulatory subunits of cAMP-dependent protein kinase (PK) called isoforms RI or RII, may differentially affect cell proliferation or

*Correspondence to A. F. De Nicola Laboratorio de Bioquímica Neuroendócrina, Instituto de Biología y Medicina Experimental, Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina.
Received 11 May 1994; accepted 12 July 1994.

differentiation [14, 15]. In agreement with these concepts, we found in pituitary tumors from F_{344} female rats increased E_2R in cell nuclei and increased binding of cAMP to the immunoprecipitable RI isoform in cytosol, whereas binding to RII—indicative of differentiation—was reduced in nuclei [16, 17]. Most studies to date, with some exceptions [7, 18], have compared biochemical changes in grown pituitary tumors with glands from hormone-free rats. In this report, we measured over a period of 1–8 weeks after diethylstilbestrol (DES) pellet implantation into male rats, some parameters of the cellular effects of estrogens, such as pituitary weight, DNA content and serum PRL. These parameters were correlated with: (1) E_2R, considering that the strict hormone-dependency of the tumor [2] relies on an activated hormone–receptor complex; (2) ornithine decarboxylase (ODC) activity, a rate-limiting enzyme in polyamine biosynthesis, which stimulates DNA, RNA and protein synthesis [19] and (3) the levels of RI and RII isoforms of cAMP-dependent PK, due to their postulated role in cell proliferation and differentiation. By carrying out a time-course analysis, we hoped to gain information regarding which parameter(s) showed the most conspicuous changes at the time of transition from normal pituitary to hyperplasia to the hormone-dependent pituitary tumor [4, 16].

MATERIALS AND METHODS

Experimental animals

Male Fischer 344 rats (200–250 g) obtained from the Atomic Energy Agency (Buenos Aires) were housed under controlled temperature and lighting conditions (lights on from 0700–1900 h) and divided in different groups in order to receive estrogenic treatment for 1–8 weeks. The treatment was carried out by implanting a single subcutaneous pellet of DES weighing 20 mg, as described previously [4, 9, 16, 17]; control rats (0 weeks) were sham implanted 1 week before sacrifice. At the time of sacrifice, animals were anesthetized with ether, bled for serum PRL determination and perfused intracardially with ice-cold 0.9% NaCl. Anterior pituitaries and DES-induced pituitary tumors (DES-T) were immediately removed from the skull of the animals, weighed on a Mettler balance, frozen on dry ice and stored at −70°C until use.

DNA content

DNA content of the glands was determined by the diphenylamine method [20], employing nuclear purified pellets sedimented after centrifugation of pituitary homogenates (see below). Results were expressed in mg/gland.

Serum PRL

Serum PRL was measured with a NIDDK rat PRL kit as shown by Becú and Libertun [21], and results were expressed in ng/ml serum, in terms of RPI standard.

E_2R

E_2R were measured in purified pituitary cell nuclei according to Roy and McEwen [22]. Tissues were homogenized in NI buffer (1 mM KH_2PO_4, 0.32 M sucrose, 3 mM $MgCl_2$, 0.25% Triton X-100, pH 6.5), and the homogenates were spun at 850 *g* for 10 min at 0–4°C. Pellets were washed twice with NII buffer (same as NI but without Triton X-100) and suspended in NIII buffer (1 mM KH_2PO_4, 2.39 M sucrose, 1 mM $MgCl_2$). Purified nuclei were prepared after centrifugation at 25,000 *g* for 30 min; the pellets were salt extracted with 0.4 M KCl and the solubilized receptors were incubated under conditions allowing for the exchange of the DES-associated receptor with [^{3}H]E_2 added to the cytosol. To this end, samples were incubated for 5 h at 25°C with 2.5 nM [^{3}H]-E_2, with a parallel set of incubations containing a 500-fold excess E_2 to determine non-specific binding. Bound and free steroids were separated on Sephadex LH 20 columns, according to Guinsburg *et al.* [23] and results of specific binding were expressed in fmol/mg DNA.

ODC activity

ODC activity was measured according to the procedure of Russell and Snyder [24]. In brief, tissues were homogenized in 50 mM KH_2PO_4 buffer (pH 7.2), 2 mM EDTA, 1 mM dithiothreitol, 30 mM NaN_3, and centrifuged at 20,000 *g* at 0–4°C. The supernatant was incubated with 0.2 mM [^{14}C]ornithine and 0.1 mM pyridoxal phosphate. After 60 min at 37°C, the reaction was stopped by addition of 40% trichloroacetic acid, and the [^{14}C]CO_2 evolved was trapped in a well containing 0.2 ml of NCS solubilizer. ODC activity was expressed in pmol [^{14}C]CO_2 released/h/mg prot [25].

Immunoprecipitation of RI and RII subunits of the cAMP-dependent PK

Immunoprecipitation was performed by methods described by Piroli *et al.* [17]. Tissues were homogenized in 50 mM sodium phosphate buffer pH 7.0 containing 100 mM NaCl, 6 mM β-mercaptoethanol, 2 mM EGTA, 0.2 mM EDTA, 10 mM $MgCl_2$, pepstatin (2 μg/ml), leupeptin (2 μg/ml), bestatin (2 μg/ml) and 1 mM benzamidine (homogenization buffer, HB). The samples were centrifuged at 105,000 *g* for 1 h at 0–4°C. Aliquots of the resulting cytosol were incubated with 0.7 pM [^{3}H]cAMP prepared in HB containing 1 mM methylisobutylxanthine (MIX), with or without a 1000-fold excess of unlabeled cAMP to determine non-specific binding. The reaction proceeded for 2 h at 25°C [26]. For immunoprecipitation, anti-RI (10 μl) or anti-RII (12 μl) antisera were added, based on immunotitration curves showing complete precipitation of [^{3}H]cAMP bound to RI or RII using these quantities [17]. The mixture was incubated for 1 h at

0–4°C, followed by addition of 5 μl of a sheep anti-rabbit 2nd antibody per μl of 1st antibody, with continuing incubation for 30 min. The resulting complexes were recovered by centrifugation at 1000 *g* for 10 min, the pellets were resuspended in 1 ml HB and filtered through GF/B glass fiber filters under vacuum. The filters were then washed twice with HB, dried in vials and their radioactive content determined after addition of 5 ml toluene–Triton X-100 (70:30, v/v) mixture. Results were expressed in pmol [^{3}H]cAMP bound to RI or to RII/mg protein, or as the RI/RII ratio.

Statistical analysis

Results were analyzed by one-way ANOVA, according to the Instat V1.0 GPIS program, followed by *post-hoc* comparisons using Duncan's test. Parameters were correlated using linear regression. The correlation coefficient (r) was calculated, and $P < 0.05$ was considered significant.

Materials

[2, 4, 6, 7-^{3}H]E_2 (98 Ci/mmol), L-[1-^{14}C]ornithine (51.6 mCi/mmol) and [2,8-^{3}H]cAMP ammonium salt (34.5 Ci/mmol) were purchased from Dupont NEN (Boston, MA). Steroids (DES, E2), cAMP, MIX, pepstatin, bestatin, benzamidine, leupeptin, EGTA, EDTA and Sephadex LH 20 were obtained from Sigma (St Louis, MO). All other reagents were of analytical grade. Policlonal rabbit antisera to RI and RII subunits were the kind gift of Dr Jack Erlichman (Department of Medicine & Biochemistry, Albert Einstein College of Medicine, Bronx, NY). The specificity of these antisera and the cross-reactivity with other species have been described previously [26, 27]. Sources of highly purified RI and RII subunits were bovine muscle and bovine cerebral cortex, respectively [27]. It has been demonstrated that anti-RII antibodies do not recognize type I holoenzyme, free RI or C subunits; similarly, anti-RI antibodies precipitate holoprotein kinase I and free RI subunit, without cross-reaction with protein kinase A II or its dissociated subunits [26]. DES pellets were kindly prepared by Dr E. Montuori (Gador Labs, Buenos Aires).

RESULTS

Estrogenization of F_{344} male rats using a pellet implantation method increased anterior pituitary weight [Fig. 1(A)]. Although weight doubled after 2 weeks of DES treatment, it was not until 4 weeks that the difference became significant with untreated rats [32.4 ± 3.2 vs 9.1 ± 0.3 mg, $P < 0.01$; Fig. 1(A)]. Pituitary growth dramatically increased between 4 and 6 weeks of treatment, reaching a plateau at 8 weeks. By this time, weight was about 70 mg, i.e. 8-fold higher than at 0 week [Fig. 1(A)].

Concomitant to weight gain, there was an increase in DNA content of the glands [Fig. 1(B)]. By 4 weeks, the increment was significant with respect to untreated rats (150.9 ± 13.4 vs 57.1 ± 2.4 μg/gland, $P < 0.01$). Between 4 and 6 weeks, DNA content continued to increase, without significative changes after that time [Fig. 1(B)]. Due to parallel changes, DNA content and pituitary weight were highly correlated [$r = 0.994$, $P < 0.0001$; Fig. 2(A)].

A raise in serum PRL was measured from the beginning of the treatment [Fig. 1(C)], continuing during the whole estrogenization period. Values as high as 3674 ± 698 ng/ml were obtained at 8 weeks [Fig. 1(C)]. Correlation of serum PRL with pituitary weight and DNA content was extremely significant [$r = 0.862$, $P < 0.0001$ and $r = 0.857$, $P < 0.0001$, respectively, Fig. 2 (B and C)].

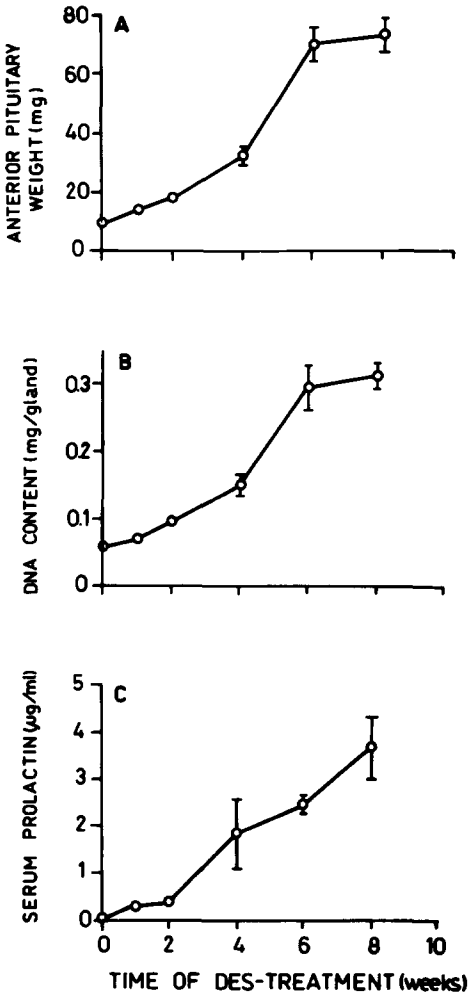

Fig. 1. Distribution of pituitary weight (A), DNA content (B) and serum prolactin (C) across time in male F_{344} rats implanted s.c. with a single 20 mg DES pellet for 1–8 weeks. Results for sham-transplanted rats (controls) are those at 0 time. Vertical lines represent the mean ± SE of $n = 4$ animals per point. The three variables became significantly higher than controls at 4 weeks or longer periods ($P < 0.01$ by ANOVA and Duncan's *post-hoc* test).

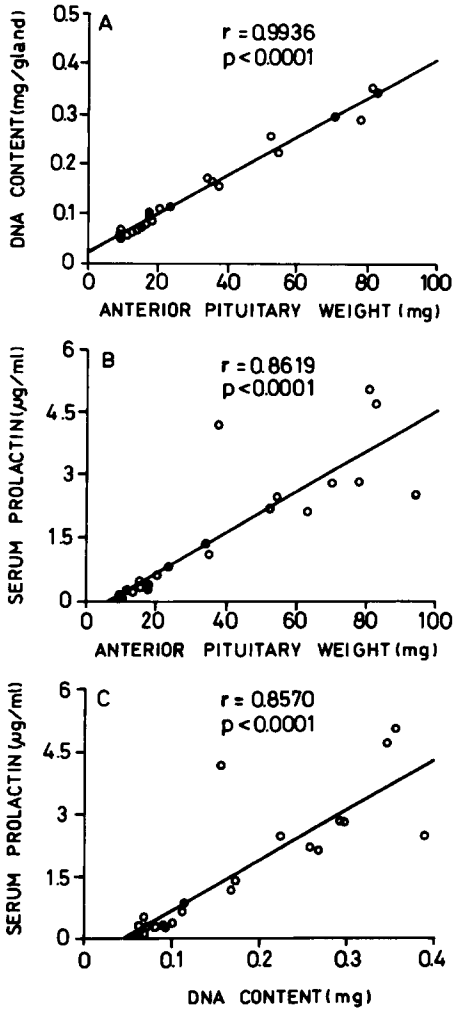

Fig. 2. Correlation analysis from data presented in Fig. 1. (A) Anterior pituitary weight vs DNA content and (B) the anterior pituitary weight vs serum PRL. A significant positive correlation was obtained (A: $r = 0.9936$, $P < 0.0001$; B: $r = 0.8619$, $P < 0.0001$). As shown in (C), DNA content and serum PRL were also significantly correlated ($r = 0.8570$, $P < 0.001$).

Figure 3(A) shows the changes observed in the content of nuclear E_2R over the 8-week period. E_2R were significantly higher than in pituitaries from untreated rats at 1 week [238.7 ± 39.0 fmol [^{3}H]E_2 bound/mg DNA vs 0 week = 44.1 ± 8.9, $P < 0.01$; Fig. 3(A)]. The highest values were recorded at 2 weeks; after this time, E_2R remained unchanged until 8 weeks, when a reduction to levels lower than those in pituitaries from 1 week-treated animals was recorded (8 weeks = 174.7 ± 9.1, $P < 0.05$ vs 1 week). However, E_2R in 8 week-treated rats was still higher than in pituitaries from untreated rats with sham implantation [8 vs 0 weeks $P < 0.01$; Fig. 3(A)].

ODC activity is shown in Fig. 3(B). The profile of ODC activity with respect to time was similar to that of E_2R, showing an increment by 1 week (103.7 ± 13.3 pmol [^{14}C]CO_2 released/h/mg protein vs 0 week = 8.9 ± 1.2, $P < 0.05$), a plateau from 2 to 6 weeks and a decrease by 8 weeks (154.8 ± 29.5 vs 6 weeks = 248.4 ± 32.1, $P < 0.05$). The correlation of ODC activity with E_2R was significant [$r = 0.702$, $P = 0.0001$; Fig. 3(C)]. However, no correlations were found between ODC activity and pituitary weight, DNA content and serum PRL (data not shown). Similarly, E_2R did not correlate with these parameters.

Our study included the measurement of immunoprecipitable RI and RII subunits of the cAMP-dependent PK over the 8-week period of DES treatment. While fluctuations in RI content were not significantly different from each other [Fig. 4(A)], RII decreased significantly by 2 weeks [1.60 ± 0.15 pmol [^{3}H]cAMP bound/mg protein vs 0 week = 2.62 ± 0.11, $P < 0.01$; Fig. 4(B)] with this trend continuing until the end of the study [8 weeks = 1.25 ± 0.26, $P < 0.01$ vs 0 week; Fig. 4(B)]. The RI/RII ratio was maintained

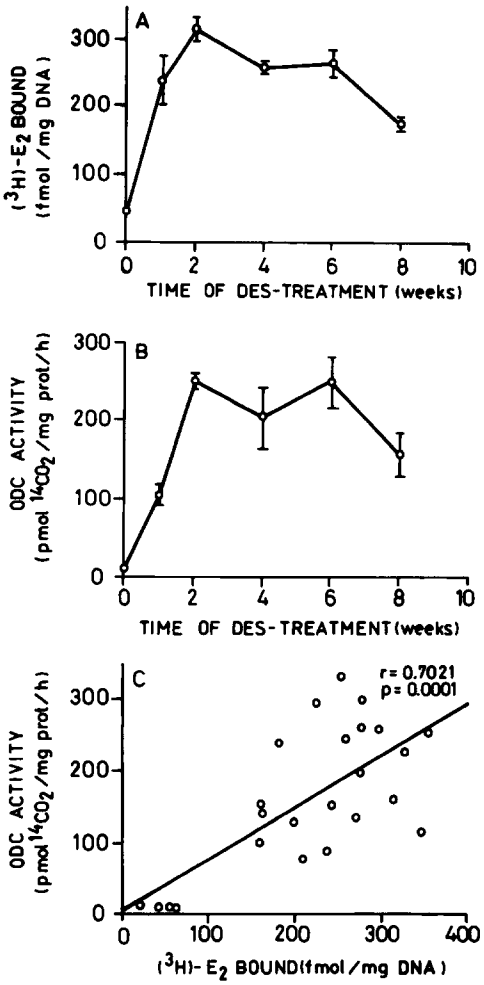

Fig. 3. Distribution of nuclear E_2R (A) and activity of ODC (B) across time in animals estrogenized during 1–8 weeks. Both variables were significantly higher than controls at all times, starting at 1 week (E_2R: $P < 0.01$; ODC: $P < 0.05$). At 8 weeks, both parameters were lower than at 1 week ($P < 0.05$). As shown in the lower graph (C), E_2R and ODC activity were significantly correlated ($r = 0.7021$, $P < 0.001$).

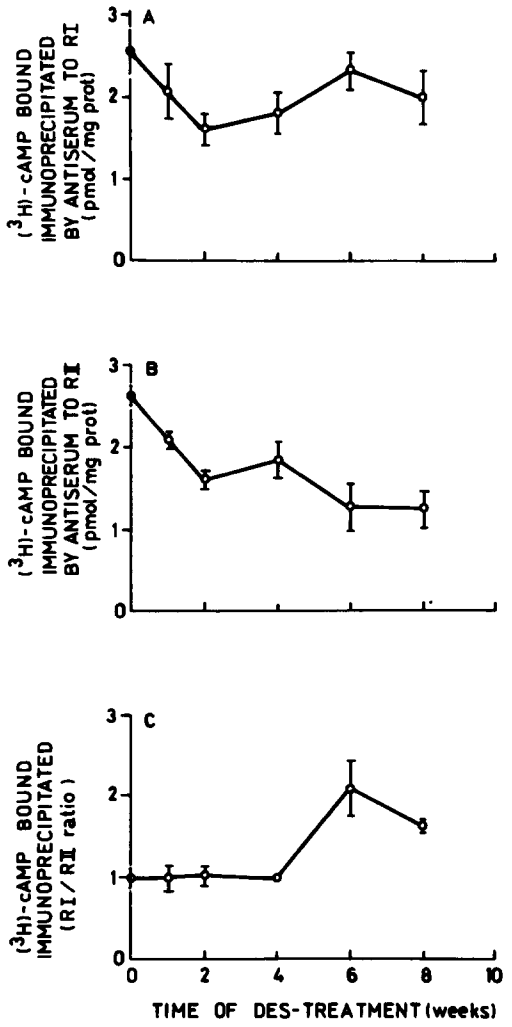

Fig. 4. Distribution of regulatory subunits of cyclic AMP dependent RI (A), RII (B) and the RI/RII ratio (C) across time in animals estrogenized for 1–8 weeks. Values for RI were not significantly different from control pituitaries. RII showed a significant reduction with time at 2 weeks or longer ($P < 0.01$). The RI/RII ratio was significantly higher than controls at 6 and 8 weeks ($P < 0.05$ or less). Statistical significance was studied by ANOVA and Duncan's *post-hoc* test.

near unity at the 1, 2 and 4 week periods, after which a significant increment was registered at 6 weeks [2.10 ± 0.35 vs 0 week $= 0.98 \pm 0.10$, $P < 0.01$; Fig. 4(C)] and was sustained at 8 weeks [1.63 ± 0.07, NS vs 6 weeks, $P < 0.05$ vs 0 week; Fig. 4(C)].

As RI content showed some stability, it did not correlate with pituitary weight or DNA content (data not shown). However, RII content negatively correlated with both pituitary weight and DNA content [$r = -0.726$, $P < 0.0001$ and $r = -0.731$, $P < 0.0001$, respectively, Fig. 5 (A and B)]. The RI/RII ratio positively correlated with the pituitary weight and the DNA content ($r = 0.755$, $P < 0.0001$ and $r = 0.819$, $P < 0.0001$, respectively [Fig. 5 (C and D)], but not with serum PRL (data not shown).

DISCUSSION

The present study was undertaken to delineate the time course of some estrogen responsive systems in the anterior pituitary undergoing tumorigenesis. Our criterion for tumor formation was a pituitary weight in excess of 50 mg, in agreement with Welsch *et al.* [28]; others [3] have established 30 mg as the limit between glandular hypertrophy and tumor. Sequentially, pituitaries exposed to estrogen first develop hypersecreting mammotrophs, with cells then becoming hypertrophic and lastly hyperplastic before the adenomatous change [4, 29, 30]. Events occurring before 4 weeks, therefore, were assigned to hypersecreting mammotrophs, then to hypertrophic/hyperplastic PRL-producing cells, whereas those in rats killed at 6 and 8 weeks to tumors, based on their secretory properties and morphologic appearance after electron microscopy [4]. Our analysis demonstrated an increase in weight and DNA content at 4–6 weeks of DES treatment, with a plateau in the 8th week. A high correlation between serum PRL and DNA content/pituitary weight was confirmed, due to the hypersecretory nature of the tissue across time [30]. Functionally, hyperplastic glands and tumors seemed to have comparable capacity to secrete PRL.

Other correlating variables were E_2R and ODC activity. Both showed abrupt increases at 1–2 weeks, plateauing thereafter and decreasing in the 8th week. Changes of E_2R could be due to increased receptor synthesis [31] or facilitated nuclear translocation of the cytoplasmic ligand-activated receptor [32]. However, it is likely that upon occupation, nuclear receptors for estrogen increase their affinity for DNA due to greater development of salt bridges between the protein and the polynucleotide [33]. The very high affinity (K_d 0.1 nM) of the E_2R found by our laboratory in estrogen-induced pituitary tumors may be due to the receptor becoming tightly bound to pituitary cell nuclei [8, 9, 34]. This mechanism could account for the E_2R increase found in normal, pre-tumorous and tumorous pituitaries. The slight, albeit significant reduction of E_2R in the 8th week, could represent receptor down-regulation due to continuous exposure to high concentrations of estrogen [35] or decreased receptor activity in mammotrophs becoming less differentiated as tumors approached their maximal growth.

As mentioned previously, ODC activity paralleled the changes in E_2R, suggesting a cause and effect phenomenon. Estrogens also induce an early increase in ODC activity in uterus, kidney, and liver [36]. The polyamines resulting from the increased ODC activity serve as intracellular mediators for hormonal trophic effects on proliferation and differentiation of cells [36, 37]. Similarly to E_2R content, ODC activity showed an early rise before significant changes in weight or DNA content, then stabilized but decayed in the 8th week period. This finding suggested an early increase but late reduction of E_2R biological activity in

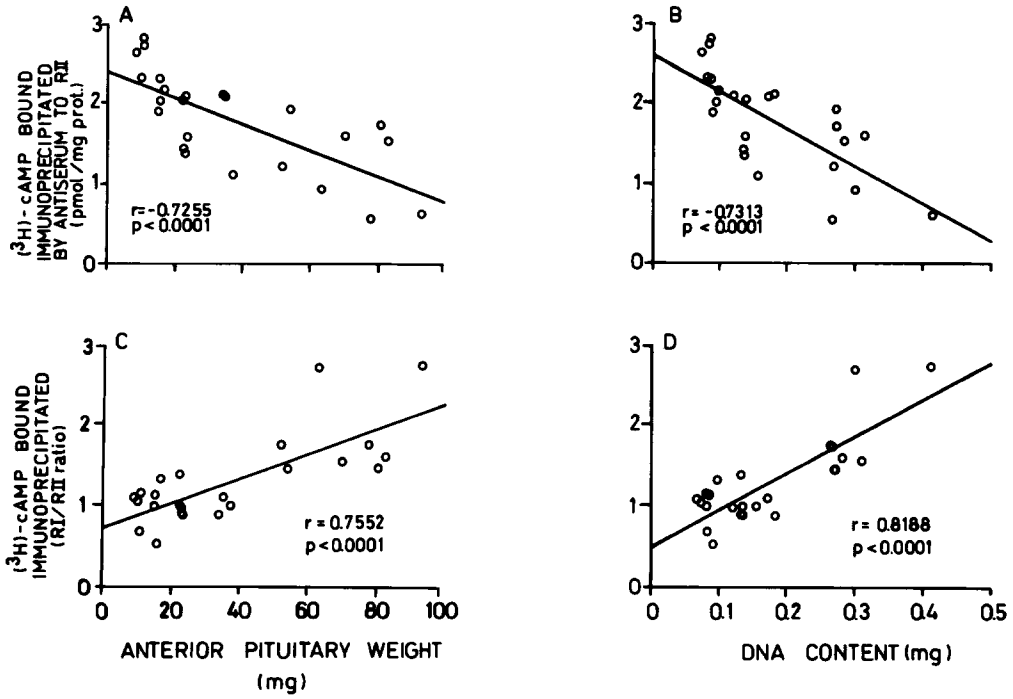

Fig. 5. Correlation analysis from data presented in Figs 1 and 4. As shown in (A) and (B), immunoprecipitated RII negatively correlated with anterior pituitary weight ($r = -0.7552$, $P < 0.0001$) and DNA content ($r = -0.7313$, $P < 0.0001$). In contrast, as shown in the lower graphs, the RI/RII ratio positively correlated with anterior pituitary weight (C; $r = 0.7552$, $P < 0.0001$) and DNA content (D; $r = 0.8188$, $P < 0.0001$), respectively.

nuclei, although it is also possible that ODC becomes more susceptible to degradation by interaction with antizyme, a specific inhibitor of ODC which is induced by polyamines [38].

The time sequence of cAMP binding by the isoforms of PK implicated this transduction pathway in the generation of anterior pituitary tumors. It is known that the cAMP-dependent PK is normally inhibited because of the tight association of the R and C subunits [39]. Whereas free C subunit is responsible for protein phosphorylation, distinct functions have recently been postulated for the dissociated RI and RII subunits. Cho-Chung *et al.* [14, 15] postulated that RI and RII subunits bound to cAMP transduce opposite signals, such as RI stimulating and RII inhibiting the control of cell proliferation. In pituitary tumors from female F_{344} rats, we observed a significant increase of [^{3}H]cAMP binding to immunoprecipitable RI without changes in nucleotide binding to RII [17]. In the current study employing male rats, binding to RI was unchanged upon hormone exposure whereas that to RII showed a time-dependent reduction. Inspection of the RI/RII ratio at the 4–8th week period suggested that upon transition from hypertrophic/hyperplastic pituitary to adenoma, there was a marked increase in this ratio. Clearly, in male rats tumor growth correlated with unbalanced expression of RI subunits (stimulatory of cell proliferation) due to a reduction in RII subunits.

In conclusion, we have observed changes in growth-promoting signals in the anterior pituitary undergoing tumorigenesis. Early following estrogenization, increments of nuclear E_2R and ODC activity preceeded the changes and may be related to pituitary growth and DNA content. Later periods presented a reduction in cAMP binding to RII subunits favoring nucleotide binding to the RI isoform, events favoring tumor formation, according to current concepts. Our hypothesis does not neglect the role of fibroblast growth factor [40], angiogenesis [41] or impaired dopaminergic hypothalamic function [6, 7] in the pathophysiology of adenomas. However, by pointing out the time sequence response of some cellular markers, it may shed light on the role of hormones in tumorigenesis.

REFERENCES

1. Selye H., Collip J. B. and Thomson D. L.: Effect of estrin on ovaries and adrenals. *Proc. Soc. Exp. Biol. Med.* 32 (1935) 1377–1381.
2. Wiklund J., Wertz N. and Gorski J. A.: A comparison of estrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzman rats. *Endocrinology* 109 (1981) 1700–1707.
3. Clifton K. H. and Meyer R. K.: Mechanism of anterior pituitary tumor induction by estrogen. *Anat. Rec.* 125 (1956) 65–81.
4. De Nicola A. F., von Lawzewitsch I., Kaplan S. E. and Libertun C.: Biochemical and ultrastructural studies on estrogen-induced pituitary tumors in F344 rats. *J. Natn. Cancer Inst.* 61 (1978) 753–763.
5. Sarkar D. K., Gottschall P. E. and Meites J.: Damage to hypothalamic dopaminergic neurons is associated with develop-

ment of prolactin-secreting pituitary tumors. *Science* **218** (1982) 684–686.
6. Di Paolo T., Daigle M. and Barden N.: Decreased tuberoinfundibular and pituitary dopamine and dihydroxyphenyl acetic acid concentration in rats with estrogen-induced pituitary tumors. *Neurosci. Lett.* **54** (1985) 251–256.
7. Morgan W. W., Steger R. W., Smith M. S., Bartke A. and Sweenery C. A.: Time course of induction of prolactin-secreting pituitary tumors with diethylstilbestrol in male rats: response of tuberoinfundibular dopaminergic neurons. *Endocrinology* **116** (1985) 17–24.
8. Weisenberg L., Piroli G., Heller C. L. and De Nicola A. F.: Binding of steroids in nuclear extracts and cytosol of rat anterior pituitary and estrogen-induced pituitary tumors. *J. Steroid Biochem.* **28** (1987) 683–689.
9. Piroli G., Grillo C., Ferrini M., Díaz-Torga G., Rey E., Libertun C. and De Nicola A. F.: Restoration by bromocriptine of glucocorticoid receptors and glucocorticoid negative feedback on prolactin secretion in estrogen-induced pituitary tumors. *Neuroendocrinology* **58** (1993) 273–279.
10. Kalberman L. E., Machiavelli G. A., De Nicola A. F., Weisenberg L. and Burdman J.: Synthesis of DNA in estrogen-induced pituitary tumors in rats: effects of bromocriptine. *J. Endocr.* **87** (1980) 221–224.
11. Durrin L. K., Weber J. L. and Gorski J.: Chromatin structure, transcription and methylation of the prolactin gene domain in pituitary tumors of Fischer F344 rats. *J. biol. Chem.* **259** (1984) 7086–7093.
12. Phelps C. and Hymer W. C.: Characterization of estrogen-induced adenohypophysial tumors in the Fischer 344 rat. *Neuroendocrinology* **37** (1983) 23–31.
13. Bodwin J. S., Mirayama P. H. and Cho-Chung Y. S.: Inverse relation between estrogen receptors and cyclic adenosine 3′,5′-monophosphate binding proteins in hormone-dependent mammary tumor regression due to dibutyril cyclic adenosine 3′,5′-monophosphate treatment. *Cancer Res.* **38** (1978) 3410–3413.
14. Cho-Chung Y. S.: Role of cyclic AMP receptor proteins in growth, differentiation and suppression of malignancy: new approaches to therapy. *Cancer Res.* **50** (1990) 7093–7100
15. Cho-Chung Y. S.: Hypothesis: Cyclic AMP and its receptor protein in tumor growth regulation *in vivo*. *J. Cycl. Nucl. Res.* **6** (1980) 163–177.
16. Piroli G., Weisenberg L., Grillo C. and De Nicola A. F.: Subcellular distribution of cyclic adenosine 3′5′-monophosphate-binding protein and estrogen receptors in control pituitaries and estrogen-induced pituitary tumors. *J. Natn. Cancer Inst.* **82** (1990) 596–601.
17. Piroli G., Pignataro O. and De Nicola A. F.: Increased activity of type I regulatory subunit of cyclic adenosine 3′,5′-monophosphate-dependent protein kinase in estrogen-induced pituitary tumors. *J. Natn. Cancer Inst.* **84** (1992) 1565–1571.
18. Lloyd H. M., Meares J. D. and Jacobi J.: Early effects of stilboestrol on growth hormone and prolactin secretion and on pituitary mitotic activity in the male rat. *J. Endocr.* **58** (1973) 227–231.
19. Russell D. H.: Ornithine decarboxylase: a key regulatory enzyme in normal and neoplastic growth. *Drug Metab. Rev.* **16** (1985) 1–88.
20. Burton K.: A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.* **62** (1965) 315–323.
21. Becú D. and Libertun C.: Comparative maturation of the regulation of prolactin and thyrotropin by serotonin and thyrotropin-releasing hormone in male and female rats. *Endocrinology* **110** (1982) 1978–1884.
22. Roy E. J. and McEwen B. S.: An exchange assay for estrogen receptors in cell nuclei of the adult rat brain. *Steroids* **30** (1977) 657–669.
23. Ginsburg M., Greenstein B. D., MacLusky N. J., Morris I. D. and Thomas P. J.: An improved method for the study of high affinity steroid binding: oestradiol binding in brain and pituitary. *Steroids* **23** (1974) 773–792.
24. Russell D. and Snyder S. H.: Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tumors. *Proc. Natn. Acad. Sci. U.S.A.* **60** (1968) 1420–1427.
25. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193** (1951) 265–275.
26. Erlichman J., Bloomgarden D., Sarkar D. and Rubin C. S.: Activation of cyclic AMP-dependent protein kinase isoenzymes: studies using specific antisera. *Archs. Biochem. Biophys.* **227** (1983) 136–146.
27. Erlichman J., Litvin Y. and Fleischer N.: Immunological and molecular characterization of the cAMP-dependent protein kinases in AtT20 cells. *J. Biol. Chem.* **259** (1984) 10289–10295.
28. Welsch C. W., Jenkins T., Amenomori Y. and Meites J.: Tumorous development of in situ and grafted anterior pituitaries in female rats treated with diethylstilbestrol. *Experientia* **27** (1971) 1350–1352.
29. Holtzman S., Stone J. P. and Shellabarger C. J.: Influence of diethylstilbestrol treatment on prolactin cells of female ACI and Sprague–Dawley rats. *Cancer Res.* **39** (1979) 779–784.
30. Stone J. P., Holtzman S. and Shellabarger C. J.: Neoplastic responses and correlated plasma prolactin levels in diethylstilbestrol-treated ACI and Sprague-Dawley rats. *Cancer Res.* **39** (1979) 773–778.
31. Walters M. R.: Steroid hormone receptors and the nucleous. *Endocrine Rev.* **6** (1985) 512–543.
32. Muldoon T. G.: Regulation of steroid hormone receptor activity. *Endocrine Rev.* **1** (1980) 339–364.
33. Skafar D. R. and Notides A.: Modulation of the estrogen receptor's affinity for DNA by estradiol. *J. Biol. Chem.* **260** (1985) 12208–12213.
34. Weisenberg L., Ortí E., Piroli G., Libertun C. and De Nicola A. F.: Hormonal effects on unoccupied estrogen receptors in nuclei of anterior pituitary glands. *Acta Physiol. Pharmac. Lat.* **36** (1986) 473–487.
35. Umans R. S., Weichselbaum R. P., Johnson C. M. and Little J. B.: Effects of estradiol concentration on levels of nuclear estrogen receptors in MCF-7 breast tumor cells. *J. Steroid Biochem.* **20** (1984) 605–609.
36. Canellakis E. S., Viceps-Madore D., Kyriadikis A. and Heller J. S.: The regulation and function of ornithine decarboxylase and of the polyamines. *Curr. Topics Cell. Regul.* **15** (1979) 155–202.
37. Tabor C. W. and Tabor H.: Polyamines. *A. Rev. Biochem.* **53** (1984) 749–790.
38. Stefanelli C., Rossoni C., Ferrari F., Flamigni F. and Caldarera C. M.: Ornithine decarboxylase and ornithine decarboxylase-inhibiting activity in rat thymocytes. *Cell Biochem. Func.* **10** (1992) 243–250.
39. Nairn A. C., Hemmings H. C. and Greengard P.: Protein kinases in the brain. *A. Rev. Biochem.* **54** (1985) 931–976.
40. Schechter J. and Weiner R.: Changes in basic fibroblast growth factor coincident with estradiol-induced hyperplasia of the anterior pituitaries of Fischer F344 and Sprague–Dawley rats. *Endocrinology* **129** (1991) 2400–2408.
41. Elias K. A. and Weiner R. I.: Inhibition of estrogen-induced anterior pituitary enlargement and arteriogenesis by bromocriptine in Fischer 344 rats. *Endocrinology* **120** (1987) 617–621.