Urokinase- and tissue-type plasminogen activators are suppressed by cortisol in the involuting prostate of castrated rats

Simon N. FREEMAN,* Paul S. RENNIE,*|| Julie CHAO,† Leif R. LUND‡ and Peter A. ANDREASEN‡§

*Department of Cancer Endocrinology, Cancer Control Agency of British Columbia, 600 West 10th Ave., Vancouver, B.C., Canada V5Z 4E6, †Department of Pharmacology, Medical University of South Carolina, Charleston, SC 29425, U.S.A.

‡Institute of Biochemistry C, University of Copenhagen, Blegdamsvej, DK-2200 Copenhagen N, Denmark,

and §Institute of Molecular Biology and Plant Physiology, University of Aarhus, 130 C.F Mollers Alle, DK-8000, Aarhus, Denmark

The effects of cortisol on the inhibition of cell-death processes and suppression of plasminogen-activator (PA) activity during involution of the rat ventral prostate gland were investigated to determine the principal type of PA activated by castration and inhibited by this hormone and whether the mechanism responsible for decreased PA activity involved reductions in enzyme synthesis or increased activity of a PA inhibitor. By using the technique of fibrin-agarose zymography, three bands of PA activity were detected at 4 and 7 days after castration: a major band with a molecular mass of approx. 30 kDa and two minor bands of 48 kDa and 64 kDa. Both the 30 kDa and 48 kDa activities were inhibited with anti-[urokinase-type PA (u-PA)] IgG. The 64 kDa activity was inhibited by anti-[tissue-type PA (t-PA)] IgG. In addition to retarding prostatic involution, daily administration of cortisol to the castrated animals suppressed all three bands of PA activity. A comparison of the pattern of total PA activity and of e.l.i.s.a. estimates of u-PA concentration during the castration-induced rise and after cortisol inhibition indicated a near perfect correlation between the two parameters. Northern-blot analysis using prostatic polyadenylated RNA revealed that the level of u-PA mRNA was highest at 4 and 7 days after castration and that cortisol treatment repressed u-PA mRNA to a level similar to that in non-castrated controls. Neither Northern hybridizations nor reverse zymography detected RNA transcripts or activity corresponding to the PA inhibitor PAI-1 in any of the prostate samples. Western-blot analysis revealed that, although the amount of arginine esterase A, another prostatic proteinase, also increased after castration, the rise in concentration of this protein was not blocked by glucocorticoid administration. Together our findings indicate the following: (1) the predominant form of PA activity induced in the prostate after castration and inhibited by cortisol is a 30 kDa form of u-PA. Although less prominent, t-PA and a 48 kDa form of u-PA follow a similar pattern of induction and inhibition; (2) changes in u-PA activity in response to castration and cortisol treatment are due to alterations in the level of u-PA mRNA and protein rather than in the activity of PAI-1; (3) not all castration-induced proteinases in the prostate are inhibited by cortisol.

INTRODUCTION

Activation of latent cell-death processes in the prostate by androgen withdrawal is an approach frequently used clinically to control metastatic prostate cancer [1,2]. This inherent capacity of the prostate gland to involute can be described in terms of an autophagic mechanism [3] which controls the differential expression of androgen-repressed genes [4–6] and the increased activity of specific hydrolytic enzymes [7–9]. With respect to the latter, plasminogen activators (PA), proteolytic enzymes which convert plasminogen into the active proteinase, plasmin, have been linked to involution of the prostate [9] as well as the involution of other secondary sexual tissues such as the uterus [10] and mammary gland [11].

Administration *in vivo* of high doses of the glucocorticoid cortisol inhibited the castration-induced rise in PA activity in the rat ventral prostate [12]. In the same set of experiments, the rate of prostatic involution was substantially retarded by treatment with this steroid hormone, supporting the hypothesis that plasminogen activators are mediators of the autophagic mechanism. Cortisol treatment of castrated rats was also found to preserve the expression of genes associated with prostatic-cell

differentiation while repressing those genes related to cell death [13]. Thus whereas androgen withdrawal triggers activation of the autophagic mechanism in the prostate, the activity of integral components of the involution process can be restrained by a non-androgenic hormone.

There are two types of mammalian PAs, the urokinase type (u-PA) and the tissue type (t-PA). They are products of different genes and appear to have different biological functions (for reviews, see [14,15]). In addition, some other proteolytic enzymes have a low plasminogen-activating [14] activity, as is the case with arginine esterase A [8,16,17]. To explore further the regulation of PA activity in the prostate by cortisol, experiments were performed to determine, first, which types of prostatic PAs were activated by castration and inhibited by this hormone and, secondly, whether the suppression of PA activity was due to reduced synthesis of the enzyme or increased activity of a PA inhibitor.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats (body wt. 250–350 g), purchased from

Abbreviations used: u- and t-PA, urokinase- and tissue-type plasminogen activator; PAI-1, plasminogen-activator inhibitor 1; PBS, phosphate-buffered saline (0.14 M-NaCl/0.003 M-KCl/0.01 M- Na_2HPO_4 /0.002 M- KH_2PO_4 , pH 7.4); poly(A)⁺, polyadenylated.

|| To whom correspondence and reprint requests should be sent.

Charles River (St. Constant, Quebec, Canada), were surgically castrated via the scrotum while the animals were under diethyl ether anaesthesia. Groups of rats were injected intraperitoneally daily for 1–7 days with either 1 ml of phosphate-buffered saline (PBS) [18] or 1 ml of PBS containing 25 mg of cortisol (sodium succinate salt; Solu-cortef; Upjohn Co. of Canada, Don Mills, Ontario, Canada).

Preparation of cytosolic extracts

As described in detail elsewhere [9], the pooled ventral prostates from three to ten rats were weighed, minced with scissors, pressed through a stainless-steel screen, Dounce-homogenized in 20 ml of 10 mM-Tes/NaOH buffer (pH 7.0)/1.5 mM-CaCl₂/0.25 M-sucrose, and centrifuged at 800 g. The supernatant was further centrifuged at 18 000 g and the final supernatant (cytosolic extract) was assayed for protein by using the Bradford method [19] and for PA activity by using the fluorescamine assay [20] as described previously [9,12].

Western-blot analysis

Samples of cytosolic extract containing 12.5 µg of protein were separated by using slab 0.1% SDS/12%-PAGE as described by Laemmli [21]. The samples were adjusted to 1% (w/v) SDS/2.5% (v/v) 2-mercaptoethanol/10% (v/v) glycerol, boiled for 5 min, and then cooled on ice. After PAGE at 200 V for 1 h, the gels were incubated for 15 min at 4 °C in transfer buffer containing 25 mM-Tris/HCl (pH 8.3)/192 mM-glycine/20% (v/v) methanol and then blotted on to nitrocellulose filters using a Bio-Rad TransBlot apparatus at 100 V for 2 h. The immunoblotting procedures using antigen overlay were as described previously [22,23]. Briefly, the nitrocellulose filters were blocked with BLOTTO [5% (w/v) dry milk in 0.01 M-phosphate (pH 7.4)/0.14 M-NaCl/1 µM-*p*-amidinophenylmethanesulphonyl fluoride/thimerosal (1 mg/ml)/NaN₃ (200 mg/l)/0.01% (v/v) antifoam] [24] for 1 h at 30 °C and then incubated with mouse anti-(arginine esterase A) monoclonal-antibody ascitic fluid (1:300 in BLOTTO) [17]. After a 3 h incubation at 30 °C with gentle shaking, the filters were washed three times with BLOTTO and then incubated with ¹²⁵I-labelled rabbit anti-mouse IgG (250 000 c.p.m./ml), which had been labelled by the lactoperoxidase method [25], for 1.5 h at 30 °C. The nitrocellulose membranes were washed three times with BLOTTO and once with PBS, dried, and exposed to Kodak X-Omat film. The relative quantity of immunoreactive esterase A was measured by densitometer scanning of autoradiograms.

Fibrin-agarose zymography

PA activity in SDS/PAGE gels was detected by fibrin-agarose zymography [26]. For zymographic analysis, SDS/PAGE was performed as described above, with the following differences: the samples contained 50 µg of protein, did not contain mercaptoethanol, and were not heat-denatured; otherwise the details of the procedure were as reported by Andreasen *et al.* [27]. In order to identify zones of lysis caused by u-PA and t-PA, antibodies raised against each of the activators were included in the fibrin-agarose gels. By using a previously published technique and immunization schedule [28], antibodies were raised against human u-PA and human t-PA that had been purified by immunoaffinity chromatography with monoclonal antibodies [29,30]. Anti-u-PA and anti-t-PA IgG, purified from serum using the Protein A-Sepharose method [31], were included in the fibrin-agarose gels at concentrations of 500 and 100 µg/ml respectively. Anti-(human u-PA) and anti-(human t-PA) IgGs were used in these experiments, since they were more readily available and since previous experience had shown that they cross-react specifically with the rat PA homologues [54–56]. As

controls, non-immune IgG was included in the gels at the same concentrations.

E.i.s.a. for u-PA

The concentration of u-PA in the cytosolic extracts was measured by a sandwich e.i.s.a. with rabbit anti-(murine u-PA) IgG [28] as the solid-phase antibody. The second layer was the same IgG preparation in affinity-purified [32] and biotin-labelled form. The third layer was peroxidase-conjugated avidin. Other details of the procedure were as described elsewhere [33]. The monospecificity of the IgG with respect to reaction with prostatic proteins was documented by Western-blot analysis (P.A. Andreasen, P. Kristensen, L. R. Lund & K. Dano, unpublished work).

Northern-blot analysis

Prostates used for RNA preparations were excised directly into liquid N₂. Polyadenylated [poly(A)⁺] RNA was prepared and analysed by using the LiCl-extraction method [35], oligo(dT)-cellulose chromatography [36] and blotting on to Nytran membranes exactly as described previously [13]. The filters were hybridized with plasmid cDNA probes labelled by nick-translation. The plasmid used as a u-PA probe, p1519, carries a 2 kb endonuclease-*Xba*I-*Sma*I fragment of murine u-PA cDNA [37]. Details of the hybridization procedure are described in [38].

RESULTS

Effects of cortisol treatment on the molecular forms of PA activity

To determine the molecular species of PAs whose activity is increased in the rat ventral prostate in response to castration and blocked by daily cortisol treatments, cytosolic extracts containing over 90% of the total cellular PA activity [9] were analysed by fibrin-agarose zymography [26]. This method is based on the fact that de-activation of u-PA and t-PA by SDS is reversible [39], and hence it provides a means of separating complex mixtures of PAs while providing an estimate of their molecular mass. The SDS/polyacrylamide gels are washed with Triton X-100 in order to remove SDS and then put in direct contact with fibrin-agarose gels containing plasminogen. Any PAs in the polyacrylamide gels diffuse into the fibrin-agarose gels and activate plasminogen to plasmin which, in turn, gives rise to position-specific clear zones of lysis in the opaque fibrin-agarose gels. The results shown in Fig. 1 indicate that prostatic extracts from rats castrated 4 (lane 3) and 7 days (lane 4) previously have at least three forms of PAs with molecular masses of approx. 64, 48 and 30 kDa. Although no zones of activity are seen in samples from non-castrated rats (lane 1), from rats castrated 1 day previously (lane 2), or from rats castrated and then treated daily with cortisol (lane 5), with prolonged exposure times a small amount of lysis was observed. The greatest amount of activity occurs in the 30 kDa region of the 7-day castrated sample. None of the zones of lysis are observed in fibrin-agarose gels in the absence of plasminogen (lane 6).

On the bases of their respective molecular masses, the 64 kDa activity probably corresponds to t-PA [40], whereas the 48 and 30 kDa activities represent enzymically active forms of rat u-PA [40,41]. In confirmation of the latter, when anti-u-PA IgG is included in the fibrin-agarose gels, the PA activity in the 48 and 30 kDa regions is inhibited (Fig. 1, lane 8); whereas pre-immune serum has no effect (lane 7). Accordingly, the major form of PA activity seen during prostatic involution is u-PA and its 30 kDa

Fig. 1. Effects of castration and cortisol treatment on the molecular forms of PA activity

Groups of three to ten rats were castrated and then injected intraperitoneally daily for 1–7 days with 1 ml of PBS (vehicle) either alone or containing 25 mg of cortisol. Prostatic extracts (18000 g supernatants; 50 µg/sample) were subjected to PAGE and fibrin-agarose zymography. Samples are as follows: from the non-castrated control (lane 1); after 1 day (lane 2), 4 days (lane 3) and 7 days following castration (lane 4); 7 days after castration and treatment with cortisol (lane 5); 7-day-castrated animals after omission of plasminogen (lane 6); inclusion of pre-immune IgG (lane 7), anti-u-PA IgG (lane 8), or anti-t-PA IgG (lane 9) in the agarose gel.

degradation product. The lysis zone at 64 kDa is inhibited when anti-t-PA IgG is included in the fibrin-agarose gel (lane 9), indicating that this activity is due to t-PA. Thus the enhanced u-PA and t-PA activities in the prostates of castrated rats are both suppressed by cortisol treatment.

Using the technique of reverse zymography [42] we were unable to demonstrate the presence of PAI-1 activity (results not shown). As a control, PAI-1 was readily detected in conditioned medium from rat hepatoma HTC cells. On the basis of experience with human PAI-1 [27], we estimate the upper limit of PAI-1 in the prostatic extracts to be approx. 20 ng/mg of protein. As previously [12], the mean wet weights of the ventral prostates in the cortisol-treated groups were about 2.5-fold higher than in the untreated 7-day castrated rats (results not shown).

Effects of cortisol on the concentration of u-PA antigen

Since u-PA activity was the predominant form of PA detected by zymography, e.l.i.s.a. was performed to determine whether the concentration of u-PA antigen is directly related to the PA activity measured in the tissue extracts. The results shown in Fig. 2 indicate that the amount of u-PA in the prostate increases as a function of time after castration. The PA activity measured in these samples (Fig. 2) is similar to that reported previously [9] and parallels almost exactly the same pattern as the e.l.i.s.a. estimate of the concentration of u-PA. Similarly, cortisol treatment suppressed both the enzyme activity and amount of u-PA in the same proportion. Regression analysis of the data reveals a near-perfect correlation ($r = 1.0$) between the two parameters. Thus, in the case of u-PA, fluctuations in activity are probably due to changes in the numbers of u-PA molecules.

Inhibition of the expression of prostatic u-PA mRNA by cortisol

Since glucocorticoids may suppress the amount of activity of u-PA either through direct repression of u-PA mRNA synthesis [43] or by the induction of a u-PA inhibitor [44], the pattern of u-PA gene expression was examined. Northern analysis was performed by using a cDNA probe for murine u-PA [37] and poly(A)⁺ RNA preparations from the prostates of non-castrated rats, rats castrated 4 and 7 days previously, and rats castrated and then treated daily for 7 days with cortisol. The results shown in Fig. 3 indicate that, at the time points examined, the highest

Fig. 2. Effects of castration and cortisol treatment on total PA activity and u-PA antigen

Prostatic extracts from groups of castrated rats were assayed for PA activity (●) by the fluorescamine assay and for u-PA antigen (○) by using e.l.i.s.a. with anti-u-PA IgG. The values for each parameter obtained after cortisol treatment of rats castrated 7 days previously are also indicated (■, PA activity; □, u-PA antigen).

Fig. 3. Expression of u-PA mRNA after castration and cortisol treatment

Poly(A)⁺ RNA (5 µg/lane), which had been isolated from the prostates of non-castrated rats (lane 1), rats castrated 4 days (lane 2) and 7 days (lane 3) previously and cortisol-treated rats castrated 7 days previously (lane 4) were analysed by Northern-blot hybridization using a ³²P-labelled u-PA cDNA probe. The size of the RNA transcript is given in kb and the amount, expressed in arbitrary absorbance units relative to that measured in the non-castrated rats being set to 1, was obtained by densitometric scanning of the autoradiogram.

concentration of u-PA transcripts in the prostate occurs 4 days after castration and declines by 7 days, a pattern differing from that of the activity profile ([9] and Fig. 2). Nevertheless, consistent with its effect on enzyme activity (Fig. 1), cortisol treatment represses u-PA expression to a level similar to that measured in non-castrated controls. Attempts to measure the expression of the PA inhibitor PAI-1 [45] by Northern analysis of the above RNA preparations failed to detect transcripts for this protein. Although in some cells glucocorticoids are known to decrease u-PA activity through induction of PAI-1 activity [43–45], this does not seem to be the principal mechanism in the rat ventral prostate. Rather, in the prostate, the high level of PA activity seen after castration and the low levels found in cortisol-

Fig. 4. Effects of castration and cortisol treatment on arginine esterase A concentration

Aliquots (12.5 µg) of prostatic extracts from groups of castrated rats and cortisol-treated rats castrated 7 days previously were separated by SDS/PAGE and Western-blotted with esterase A antibody 5A₁₀ using the antigen-overlay technique. Samples are as follows: from the non-castrated control (lane 1); after 1 day (lane 2), 4 days (lane 3), 7 days (lane 4), and 10 days (lane 5) after castration; 7 days after castration with daily treatment with cortisol (lane 6).

treated animals can be more easily accounted for by parallel changes in the relative concentration of u-PA mRNA.

Effects of castration and cortisol treatment on arginine esterase A

In addition to u-PA and t-PA, arginine esterase A is another proteinase found in the prostate which has the capacity to activate plasminogen [8,16,17], although with a much lower efficiency than u-PA or t-PA. To investigate whether this enzyme is also induced by castration and inhibited by glucocorticoids, cytosolic extracts from the prostates of non-castrated, castrated and cortisol-treated rats were analysed by Western blotting for the relative levels of arginine esterase A using the highly sensitive ¹²⁵I-antigen-overlay technique [23] together with a specific monoclonal antibody (5A₁₀) [17] to arginine esterase A. The immunoblot in Fig. 4 reveals one predominant band with a molecular mass of approx. 32 kDa, which, although present in the sample from non-castrated animals (lane 1), increases in intensity as a function of the interval after castration (lanes 2–5). However, unlike u-PA and t-PA, the castration-induced rise in this protein is not blocked by glucocorticoid administration (lane 6). Thus the retarding action of cortisol on prostatic involution and u-PA synthesis does not extend to all castration-induced proteinases.

DISCUSSION

The involution of the prostate gland is the end result of a complex process involving a cascade of molecular events triggered by de-repression of androgen-regulated genes governing as-yet-unknown components of programmed cell death [4–7]. We previously demonstrated that a rise in prostatic PA activity is closely correlated with the autophagic process [9] and that prostatic involution and PA activity were both inhibited in parallel by cortisol administration [12]. In the present study we found that both types of PAs, u-PA and t-PA, were elevated in the prostate after castration, with the 30 kDa form of u-PA the most prominent form detected (Fig. 1). Cortisol treatment

reduced the level of both types of activator activity back to that of non-castrated controls (Fig. 1). By comparison, arginine esterase A, another prostatic proteinase with some associated PA activity [8,16,17], also increased after castration; however, the rise in concentration of this enzyme was not blocked by glucocorticoid administration (Fig. 4).

In the case of u-PA, a sandwich e.l.i.s.a. was used to demonstrate that the amount of antigenic u-PA present in the prostatic extracts closely paralleled the amount of PA activity measured (Fig. 2) and that changes in the level of u-PA mRNA followed a similar pattern (Fig. 3). Since neither PAI-1 activity nor the presence of PAI-1 mRNA could be detected in the gland, it would seem that this PA inhibitor is not a major contributor to modulations in u-PA activity. Together our results imply that the castration-induced rise in u-PA activity in the prostate is due to synthesis *de novo*, which, in turn, is inhibited by glucocorticoid administration. Although glucocorticoid regulation of u-PA and t-PA has been studied *in vitro* with a number of cell lines (for a review, see [14]), to our knowledge this is the first report on glucocorticoid regulation of these activators *in vivo*.

u-PA has been implicated in a wide variety of cellular processes, including cell migration, tumour invasiveness and metastasis, tissue remodelling and organ involution, whereas t-PA performs a more restricted function and is active primarily in fibrinolysis and, to a lesser extent, in prohormone processing and ovulation [14,15]. The present investigation has demonstrated a close link between the occurrence of these two activators and castration-induced involution, although the precise role that each activator has in the involution process still needs to be determined. It is anticipated that immunocytochemical localization of the activators and specific inhibition of their activity in the prostate *in vivo* may provide valuable information in that respect.

Although the precise mechanism through which cortisol inhibits u-PA and t-PA synthesis is unknown, it is likely that it is of restricted specificity and probably mediated through the glucocorticoid receptor. With respect to specificity, cortisol, in the present experiments, did not prevent the rise in esterase A after castration (Fig. 4). In another study [13] using the same experimental protocol, cortisol was found to down-regulate glucocorticoid-receptor gene expression and to reduce the expression of TRPM-2, a marker of prostatic epithelial-cell death [4,46], but was unable to mimic the action of androgens in down-regulating the androgen-receptor gene. This latter finding, together with the observations that cortisol has an almost negligible affinity for the androgen receptor [47] and does not maintain or elevate the nuclear concentration of dihydro-testosterone in the prostate [12], make it unlikely that cortisol possesses androgenic activity. Rather, there are examples of dual control by glucocorticoids and androgens: these include stimulation of the growth of the 'androgen-dependent' Shionogi carcinoma [48] and the mutual interaction of their receptors with hormone-response elements of the MMTV (mouse mammary tumour virus) genome [49]. Accordingly it is reasonable that both androgen receptors and glucocorticoid receptors are able to bind to hormone-responsive elements of the u-PA gene and independently repress its expression.

There are certain clinical implications inherent to the finding that cortisol can regulate the expression of genes associated with prostatic differentiation and cell death. In prostatic carcinomas treated by androgen-withdrawal therapy, glucocorticoids on the one hand may substitute for androgens to preserve the androgen-dependence of tumours in a manner analogous to that seen in the Shionogi carcinoma [50,51], whereas, on the other hand, they may compromise the extent of tumour regression through interference with the de-repression of the u-PA gene.

Paradoxically, increased levels of u-PA are characteristic of some prostatic tumours [52], particularly those that metastasize to the bone [53]. Thus the efficacious application of glucocorticoids in the treatment of prostate cancer may require a careful evaluation of the stage of the disease and the appropriate steroid dosage in order that the best compromise between promoting tumour regression and suppressing tumour metastasis can be achieved.

Dr. D. Belin is thanked for the gift of u-PA probe and Dr. T. Ny for the t-PA probe. The excellent technical assistance of Ms. Bewte Johannessen is gratefully acknowledged. This research was supported by grants from the National Cancer Institute of Canada (to P.S.R.), the Danish Medical Research Council and the Danish Cancer Society (to P.A.A.) and the National Institute Health (HL-29397) (to J.C.).

REFERENCES

- Neumann, F. (1983) in *Androgens and Antiandrogens* (Schroder, F. H., ed.), pp. 97–111, Schering Nederland BV, Weesp, The Netherlands
- Goldenberg, S. L., Bruchovsky, N., Rennie, P. S. & Coppin, C. M. (1988) *J. Urol.* **140**, 1460–1465
- Bruchovsky, N., Lesser, B., Van Doorn, E. & Craven, S. (1975) *Vitam. Horm. (N.Y.)* **33**, 61–102
- Monpetit, M. L., Lawless, K. R. & Tenniswood, M. (1986) *Prostate* **8**, 25–36
- Quarumby, V. E., Beckman, W. C., Jr., Wilson, E. M. & French, F. S. (1987) *Mol. Endocrinol.* **1**, 865–874
- Buttayan, R., Zakeri, Z., Lockshin, R. & Wolgemuth, D. (1988) *Mol. Endocrinol.* **2**, 650–657
- Tanabe, E. T., Lee, C. & Grayhack, J. T. (1982) *J. Urol.* **127**, 826–828
- Frenette, G., Dube, J. Y. & Tremblay, R. R. (1983) *Prostate* **4**, 383–390
- Rennie, P. S., Bouffard, R., Bruchovsky, N. & Cheng, H. (1984) *Biochem. J.* **221**, 171–178
- Shimada, H., Okamura, H., Espey, L. L. & Mori, T. (1985) *J. Endocrinol.* **104**, 295–298
- Ossowski, L., Biegel, D. & Reich, E. (1979) *Cell (Cambridge, Mass.)* **16**, 929–940
- Rennie, P. S., Bowden, J.-F., Bruchovsky, N. & Cheng, H. (1988) *Biochem. J.* **252**, 759–764
- Rennie, P. S., Bowden, J.-F., Freeman, S. N., Bruchovsky, N., Cheng, H., Lubahn, D. B., Wilson, E. M., French, F. S. & Main, L. (1989) *Mol. Endocrinol.* **3**, 703–708
- Dano, K., Andreasen, P. A., Grondahl-Hansen, J., Kristensen, P., Nielsen, L. S. & Skriver, L. (1985) *Adv. Cancer Res.* **44**, 139–266
- Andreasen, P. A., Georg, B., Lund, L. R., Riccio, A. & Stacy, S. (1990) *Mol. Cell. Endocrinol.*, in the press
- Ryan, T. J., Keegan, M. C., McMartin, D. N. & Dickerman, H. W. (1988) *Biochim. Biophys. Acta* **800**, 87–95
- Chao, J. & Chao, L. (1987) *Biochim. Biophys. Acta* **910**, 233–239
- Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167–182
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Kessner, A. & Troll, W. (1976) *Arch. Biochem. Biophys.* **176**, 411–416
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lammle, B., Berrettini, M. & Griffin, J. H. (1986) *Anal. Biochem.* **156**, 118–125
- Chao, S., Chao, L. & Chao, J. (1989) *Biotechniques* **7**, 68–71
- Johnson, D. A., Gautsch, J. W., Spasman, J. D. & Elder, J. H. (1984) *Gene Anal. Tech.* **1**, 3–8
- Shimamoto, K., Chao, J. & Margolius, H. S. (1980) *J. Clin. Endocrinol. Metab.* **51**, 840–848
- Granelli-Piperno, A. & Reich, E. (1978) *J. Exp. Med.* **148**, 223–234
- Andreasen, P. A., Nielsen, L. S., Kristensen, P., Grondahl-Hansen, J., Skriver, L. & Dano, K. (1986) *J. Biol. Chem.* **261**, 7644–7651
- Dano, K., Nielsen, L. S., Moller, V. & Engelhart, M. (1980) *Biochim. Biophys. Acta* **630**, 146–151
- Nielsen, L. S., Hansen, J. G., Skriver, L., Wilson, E. L., Kaltoft, K., Zeuthen, J. & Dano, K. (1982) *Biochemistry* **24**, 6410–6415
- Nielsen, L. S., Hansen, J. G., Andreasen, P. A., Skriver, L., Dano, K. & Zeuthen, J. (1983) *EMBO J.* **2**, 115–119
- Hjelm, H., Hjelm, K. & Sjoquist, J. (1972) *FEBS Lett.* **28**, 73–76
- Larsson, L.-I., Skriver, L., Nielsen, L. S., Grondahl-Hansen, J., Kristensen, P. & Dano, K. (1984) *J. Cell Biol.* **98**, 894–903
- Nielsen, L. S., Grondahl-Hansen, J., Andreasen, P. A., Skriver, L., Zeuthen, J. & Dano, K. (1986) *J. Immunoassay* **7**, 209–228
- Reference deleted
- Cathala, G., Savouret, J., Munday, B., West, B. L., Karin, M., Martial, J. A. & Baxter, J. D. (1980) *DNA* **2**, 329–335
- Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1408–1413
- Belin, D., Vassalli, J.-D., Combepine, C., Godeau, F., Nagamine, Y., Reich, E., Kacher, H. P. & Duvoisin, R. M. (1985) *Eur. J. Biochem.* **148**, 225–232
- Lund, L. R., Riccio, A., Andreasen, P. A., Nielsen, L. S., Kristensen, P., Laiho, M., Saksela, O., Blasi, F. & Dano, K. (1987) *EMBO J.* **6**, 1281–1286
- Unkeless, J. C., Dano, K., Kellerman, G. M. & Reich, E. (1974) *J. Biol. Chem.* **249**, 4295–4305
- Dano, K. & Reich, E. (1978) *J. Exp. Med.* **147**, 745–757
- Martikainen, P., Suominen, J. & Vihko, K. (1989) *Acta Endocrinol.* **120**, 43–49
- Erickson, L. A., Lawrence, D. A. & Loskutoff, D. J. (1984) *Anal. Biochem.* **137**, 454–463
- Busso, N., Belin, D., Failly-Crepin, C. & Vassalli, J.-D. (1986) *J. Biol. Chem.* **261**, 9309–9315
- Seifert, S. C. & Gelehrter, T. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6130–6133
- Andreasen, P. A., Christensen, T. H., Huang, J.-Y., Nielsen, L. S., Wilson, E. L. & Dano, K. (1986) *Mol. Cell. Endocrinol.* **45**, 137–147
- Leger, J. G., Monpetit, M. L. & Tenniswood, M. P. (1987) *Biochim. Biophys. Res. Commun.* **147**, 196–203
- Wilson, E. M. & French, F. S. (1976) *J. Biol. Chem.* **251**, 5620–5629
- Omukai, Y., Nakamura, N., Hiraoka, D., Nishizawa, Y., Uchida, N., Noguchi, S., Sato, B. & Matsumoto, K. (1987) *Cancer Res.* **47**, 4329–4334
- Parker, M. G., Webb, P., Needham, M., White, R. & Ham, J. (1987) *J. Cell Biochem.* **35**, 285–292
- Darbre, P. D. & King, R. J. B. (1987) *Cancer Res.* **47**, 2937–2944
- Darbre, P. D. & King, R. J. B. (1988) *J. Cell. Biochem.* **36**, 83–89
- Camiolo, S. M., Markus, G., Englander, L. S., Siuta, M. R., Hobika, G. H. & Kohga, S. (1984) *Cancer Res.* **44**, 311–318
- Kirchheimer, J. C., Pfluger, H., Ritschl, P., Hienert, G. & Binder, B. R. (1985) *Invasion Metastasis* **5**, 344–355
- Kristensen, P., Nielsen, L. S., Grondahl-Hansen, J., Andreasen, P. A., Larsson, L.-I. & Dano, K. (1985) *J. Cell. Biol.* **101**, 305–311
- Kristensen, P., Nielsen, J. H., Larsson, L.-I. & Dano, K. (1987) *Endocrinology (Baltimore)* **121**, 2238–2244
- Kristensen, P., Hougard, D. M., Larsson, L.-I. & Dano, K. (1987) *Histochemistry* **85**, 431–437

Received 13 November 1989/6 February 1990; accepted 12 February 1990