Glucocorticoid and Progesterone Inhibit Involution and Programmed Cell Death in the Mouse Mammary Gland

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Abstract. Milk production during lactation is a consequence of the suckling stimulus and the presence of glucocorticoids, prolactin, and insulin. After weaning the glucocorticoid hormone level drops, secretory mammary epithelial cells die by programmed cell death and the gland is prepared for a new pregnancy. We studied the role of steroid hormones and prolactin on the mammary gland structure, milk protein synthesis, and on programmed cell death. Slow-release plastic pellets containing individual hormones were implanted into a single mammary gland at lactation. At the same time the pups were removed and the consequences of the release of hormones were investigated histologically and biochemically. We found a local inhibition of involution in the vicinity of deoxycorticosterone- and progesterone-release pellets while prolactin-release pellets were ineffective. Dexamethasone, a very stable and potent glucocorticoid hormone analogue, inhibited involution and programmed cell death in all the mammary glands. It led to an accumulation of milk in the glands and was accompanied by an induction of protein kinase A, AP-1 DNA binding activity and elevated c-*fos*, *jun*B, and *jun*D mRNA levels. Several potential target genes of AP-1 such as stromelysin-1, c-*jun*, and SGP-2 that are induced during normal involution were strongly inhibited in dexamethasone-treated animals. Our results suggest that the cross-talk between steroid hormone receptors and AP-1 previously described in cells in culture leads to an impairment of AP-1 activity and to an inhibition of involution in the mammary gland implying that programmed cell death in the postlactational mammary gland depends on functional AP-1.

Development of the mammary gland is characterized by distinct phases of cellular proliferation during puberty and pregnancy, differentiation of epithelial cells in late pregnancy and extensive cell death and tissue remodeling during postlactational involution. It was shown that during involution the majority of secretory epithelial cells but also myoepithelial cells die by apoptosis, a form of programmed cell death (PCD)[1], (Walker et al., 1989; Strange et al., 1992; Bielke et al., 1995). The phases of mammary epithelial cell proliferation and differentiation were shown to be under stringent hormonal control (Topper and Freeman, 1980). In culture, insulin, prolactin, and hydrocortisone are able to maintain mammary explants in a state of lactation (Topper et al., 1975). Furthermore, these three hormones were shown to be sufficient to induce differentiation of established mammary epithelial cells in vitro (Danielson et al., 1984; Doppler et al., 1989; Reichmann et al., 1989). That some of these hormones also have a critical function in the maintenance of lactation in vivo is substantiated by the observation that hydrocortisone (Johnson and Meites, 1958; Ossowski et al., 1979) and prolactin (Ossowski et al., 1979) inhibited mammary gland regression when injected at high doses into rodents. The mechanism by which these hormones inhibit involution is not known and it was not clear from these studies whether prolactin and hydrocortisone act directly or indirectly on mammary cells in vivo. AP-1 is a heterogeneous transcription factor consisting of a dimeric complex of members of the Fos, Jun, and ATF/CREB families of proteins (Benbrook and Jones, 1990; Angel and Karin, 1991; Hai and Curran, 1991). Recently, we have shown that AP-1 is induced during involution of the mouse mammary gland (Marti et al., 1994*a*). Members of AP-1 such as c-Fos and JunD are expressed in the epithelium where they may act as potential regulators of epithelial cell death (Marti et al., 1995). Indeed, several genes have been re-

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1. *Abbreviations used in this paper*: AP-1, activator protein 1; PCD, programmed cell death; PKA, protein kinase A; SGP-2, sulfated glycoprotein 2; TIMP-1, tissue inhibitor of metalloproteases 1; WAP, whey acidic protein.

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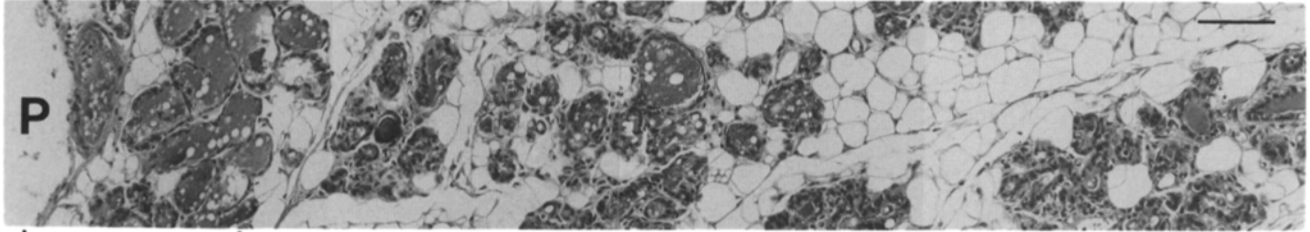

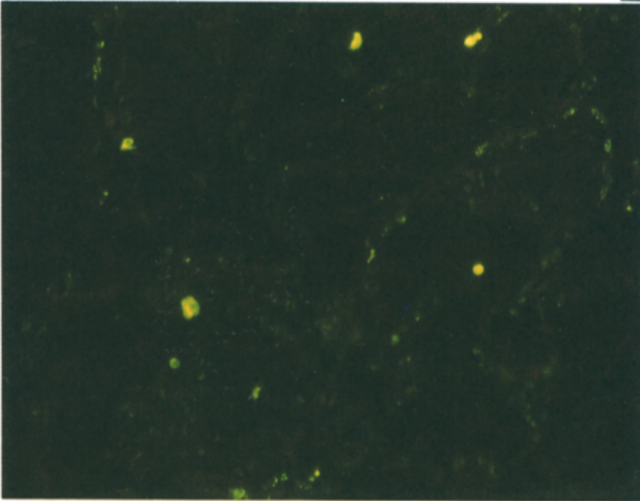

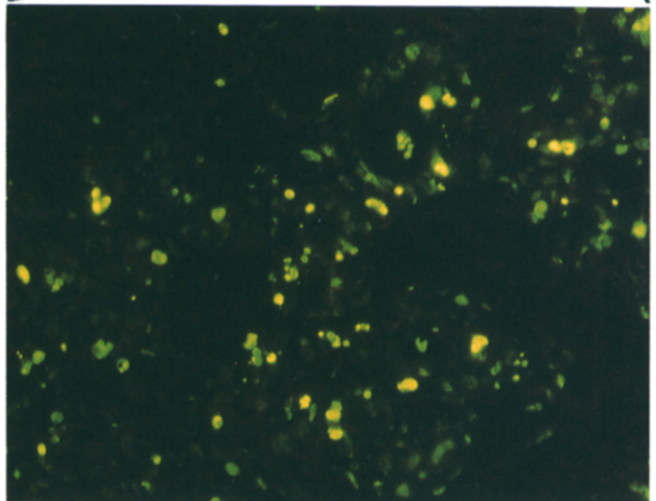

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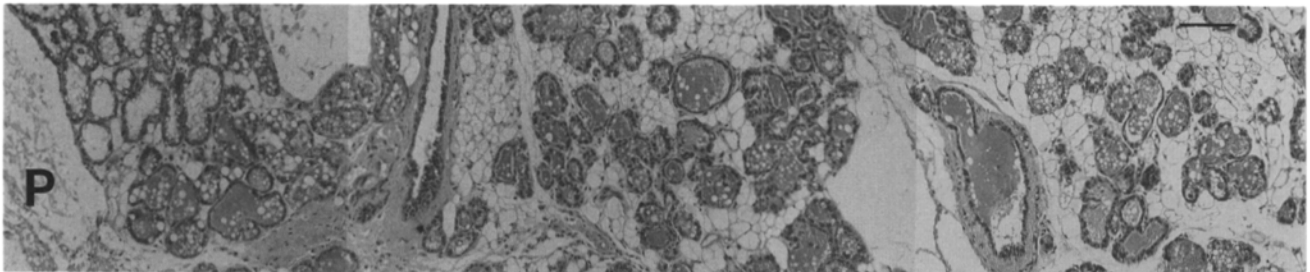
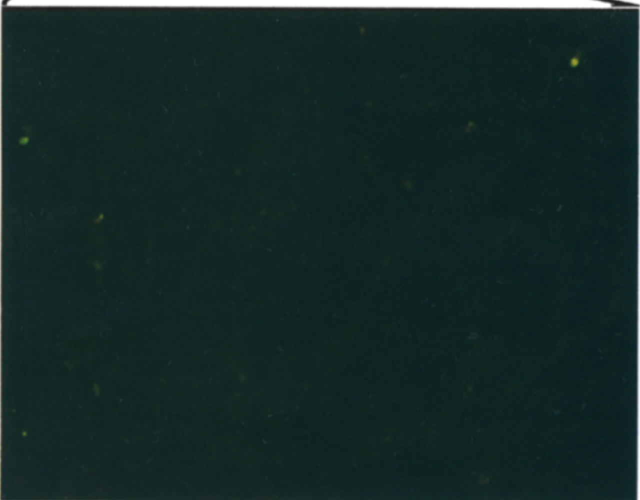
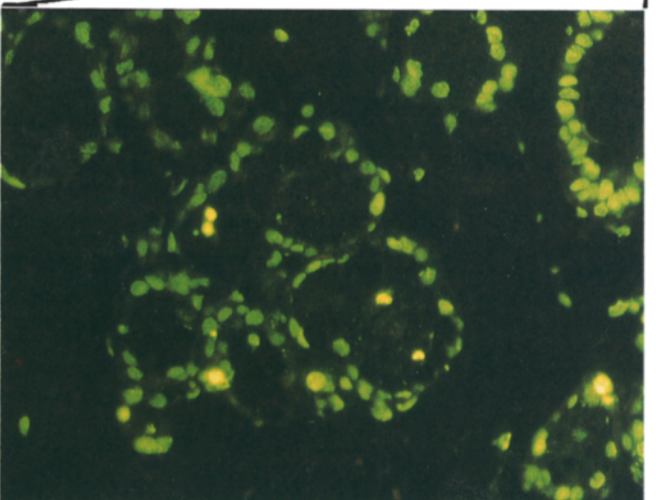






ported to be induced during mammary involution that are potential target genes of AP-1. Among those genes are sulfated glycoprotein (SGP-2) (Strange et al., 1992), plasminogen activator (Ossowski et al., 1979), stromelysin-1 (McDonnell et al., 1990; Kerr et al., 1992; Strange et al., 1992), and c-*jun* (Marti et al., 1994*a*). In addition, several reports demonstrate an involvement of c-Fos (Colotta et al., 1992; Smeyne et al., 1993) or c-Jun (Colotta et al., 1992; Estus et al., 1994) in the regulation of apoptosis of lymphocytes, fibroblasts, or nerve cells in culture. We and others have described a complex cross-talk between AP-1 and several members of the steroid receptor superfamily (Jonat et al., 1990; Lucibello et al., 1990; Schüle et al., 1990; Yang-Yen et al., 1990; Shemshedini et al., 1991; Touray et al., 1991*a,b*; Marti et al., 1994*b*). In these studies it was shown that ligand activated glucocorticoid receptor impairs c-Fos and c-Jun activity by a mechanism that probably involves a direct protein/protein interaction (Jonat et al., 1990; Touray et al., 1991*a*). Other steroids such as retinoic acid (Schüle et al., 1991; Fanjul et al., 1994; Herrlich and Ponta, 1994) and progesterone (Shemshedini et al., 1991) were shown to similarly impair the activity of c-Fos and c-Jun (in the case of retinoic acid) or c-Jun (in the case of progesterone). The cross-talk between the glucocorticoid receptor and AP-1 is mutual in that c-Fos, c-Jun, and JunD are blocked by ligand activated glucocorticoid receptor and, vice versa, glucocorticoid receptor function is impaired by overexpressed c-Fos and c-Jun (but not JunD) (Schüle et al., 1990; Yang-Yen et al., 1990; Touray et al., 1991*a*; Berko-Flint et al., 1994; Marti et al., 1994*b*). Most of these studies were carried out with cells in culture and only a limited number of reports exists that describes this cross-talk in vivo (Herrlich and Ponta, 1994); e.g., tumor promotion in the mouse skin that is dependent on a continuous stimulation of AP-1 function by phorbol esters was shown to be strongly inhibited by dexamethasone or retinoic acid (Belman and Troll, 1972; Verma, 1987). Furthermore, the response of embryonal retina glial cells towards cortisol was shown to be blocked by phorbol esters (Berko-Flint et al., 1994). In this study we demonstrate that glucocorticoid and progesterone inhibit mammary gland involution by a local effect and we show that AP-1 is a potential target of this glucocorticoid hormone-mediated inhibition. We provide evidence that an absence of PCD of epithelial cells coincides with an inhibition of expression of several potential AP-1–dependent target genes including stromelysin-1, SGP-2, and c-*jun*.

Material and Methods

Preparation of Implants

Dexamethasone, deoxycorticosterone acetate, and progesterone (Sigma Chem. Co., St. Louis, MO) were dissolved in 70% ethanol and prolactin (Sigma) was dissolved in water. Steroid hormones and prolactin solutions were mixed with BSA and lyophilized. 10% Elvax dissolved in methylene chloride was added and the mixture was again lyophilized. Pellets were cut to a size of ~1.0 mg. One pellet contained 0.5 mg BSA in combination with 60 μg steroid hormones or 50 μg prolactin. Control pellets contained 0.5 mg BSA.

Manipulation of Animals and Implantation of Elvax Pellets

Tissue was prepared from the fourth inguinal mammary gland of MORO mice. Involution was induced after 5 d of lactation by removing the pups. Lactating animals were anesthesized by intraperitoneal injection of Nembutal (50 μg/g body weight) and Elvax pellets containing deoxycorticosterone acetate, progesterone, dexamethasone, prolactin, dexamethasone plus prolactin or BSA alone were implanted into one of the fourth inguinal mammary glands by cutting a small pocket with an iris scissors. Implanted and matched glands were analyzed 2, 3, or 4 d later. Blood was collected from the carotid arteria of anesthesized animals and analyzed for serum glucocorticoid levels.

Histological Analysis and Terminal Transferase Reaction

Tissue samples were fixed in freshly prepared 4% formaldehyde in phosphate-buffered saline (50 mM KH_2PO_4, 150 mM NaCl, pH 7.4), embedded in paraffin and 5-μm thick sections were stained with hematoxylin and eosin. For the terminal transferase reaction the procedure of Gavrieli et al. (1992) was employed with several modifications. Sections were treated with proteinase K (10 ng/ml) for 15 min at 25°C. Digoxigenin-labeled dUTP (Boehringer Mannheim Corp., Indianapolis, IN) was incorporated in the terminal transferase reaction and subsequently detected using fluorescein-coupled Fab fragments (Boehringer Mannheim Corp.).

DNA Fragmentation Analysis

DNA fragmentation was analyzed as described (Ishida et al., 1992) with several modifications. 10–20 mg tissue was collected in cold PBS, homogenized with a polytron and incubated for 15 m on ice in 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, and 0.2% Triton X-100. The lysate was centrifuged and the supernatant that contained RNA and small DNA fragments (but not intact chromatin) was extracted once with phenol and once with phenol/chloroform. The aqueous phase was adjusted to 300 mM NaCl and precipitated in 2 vol of ethanol. The nucleic acid was pelleted, air dried and dissolved in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. After RNase A treatment 20 μg DNA was electrophoresed in a 1.5% agarose gel with Boyer's buffer (50 mM Tris-HCl, pH 8, 20 mM NaAc, 2 mM EDTA, 18 mM NaCl) containing ethidium bromide and visualized on an UV transilluminator.

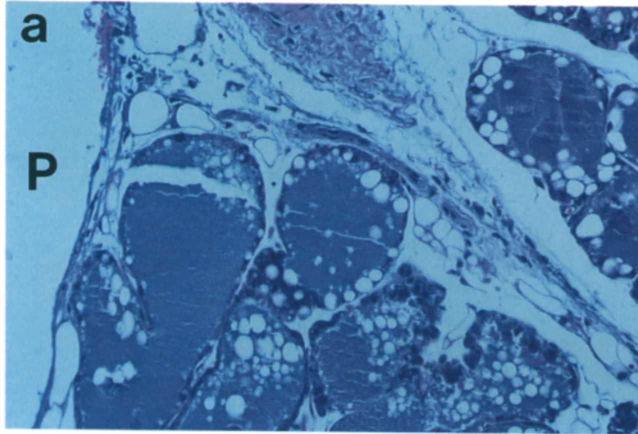
RNA Analysis

Total RNA was prepared using guanidinium thiocyanate as described (Chomczynski and Sacchi, 1987). 10 μg total or 5 μg poly(A)-selected RNA were separated electrophoretically, transferred to nitrocellulose, and probed with random-prime labeled cDNA fragments corresponding to homologous sequences. Total RNA was used to probe for WAP and SGP-2 expression, and poly(A)-selected RNA to probe for c-*fos*, c-*jun*, *jun*B, *jun*D, stromelysin-1, TIMP-1, and actin expression.

Nuclear Extract Preparation, PKA Assay, and DNA Bandshift Analysis

Nuclear extracts were prepared as described (Marti et al., 1994*a*). For PKA assays 5 μg extract was incubated with a fluorescent peptide (Kemptide; Promega Corp., Madison, WI) following the instructions of the manufacturer. Phosphorylated peptide was separated from nonphosphorylated peptide by agarose gel electrophoresis and visualized on an UV transilluminator.

Figure 1. Local inhibition of involution and PCD by deoxycorticosterone acetate and progesterone. Deoxycorticosterone acetate-release pellets (*a*) or progesterone-release pellets (*b*) were implanted at lactation, the pups were removed and the glands were analyzed 3 d later. The upper panels show an overview of a hematoxylin/eosin stained section adjacent to the implanted pellet (*P*). The lower panels show the indicated areas after terminal transferase assay reactions of serial sections at higher magnification. Bar, 100 μm.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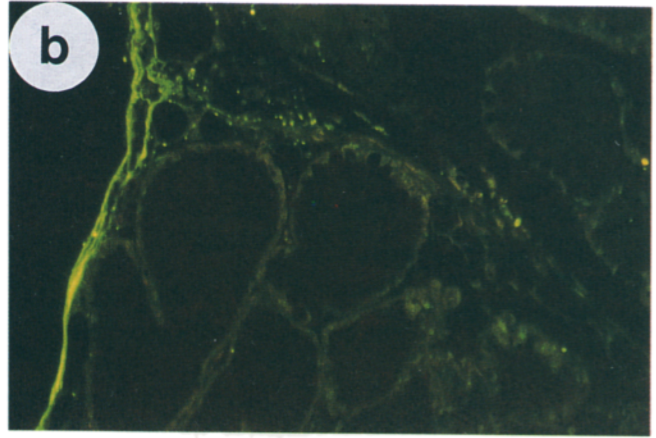
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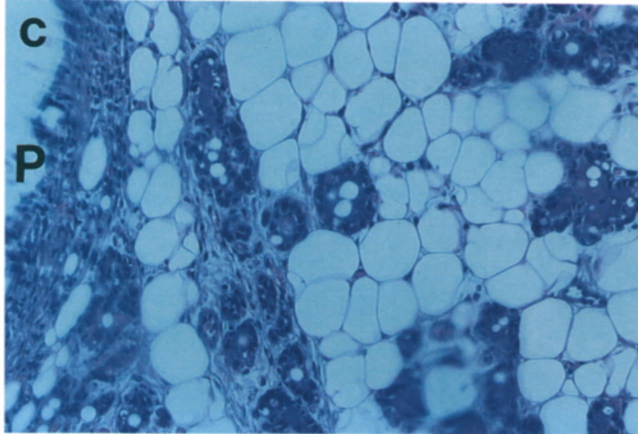
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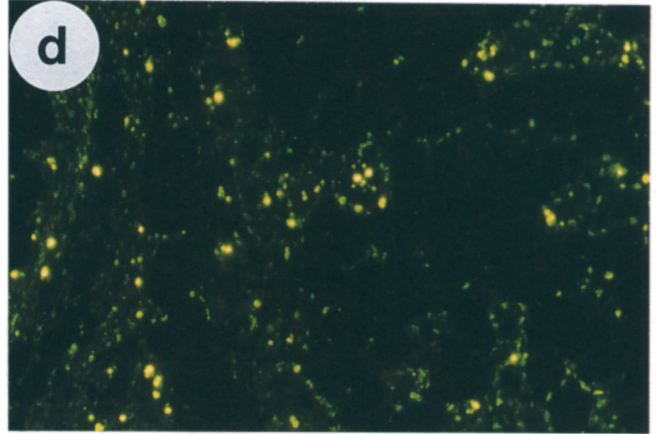
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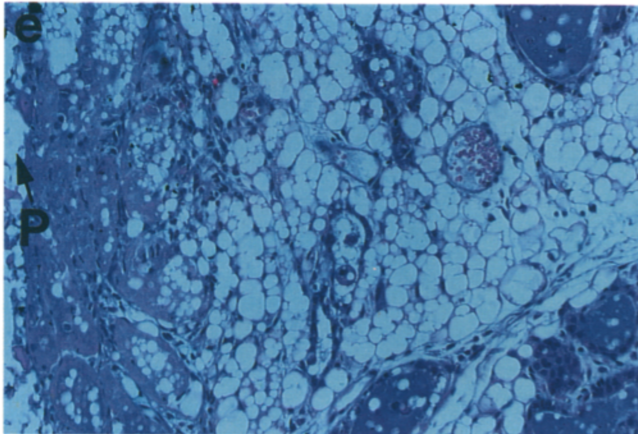
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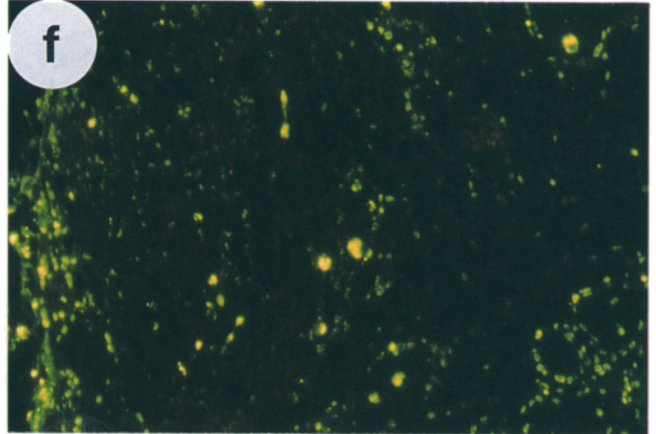
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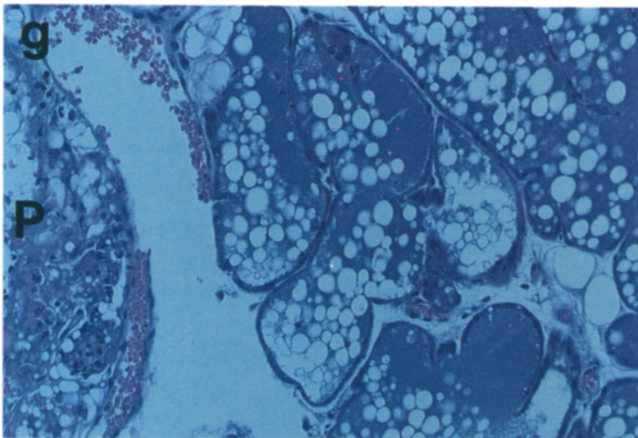
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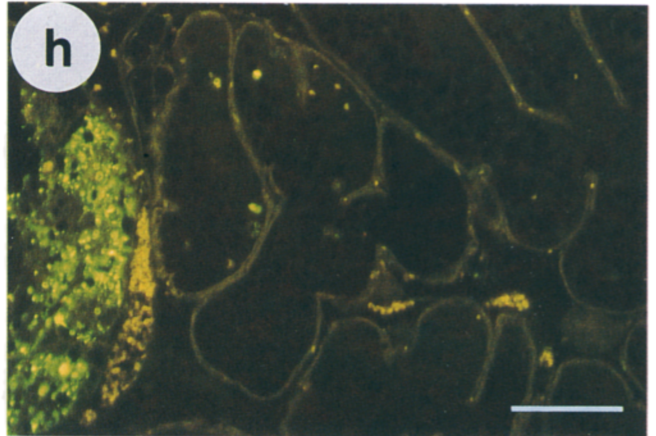
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Electrophoretic mobility shift assays were performed as described (Marti et al., 1994*a*). ^{32}P-labeled double-stranded oligonucleotides specific for AP-1 (5' AAGCATGAGTCAGACAC 3') was incubated with nuclear extract (5 μg) in a final volume of 25 μl 5 mM $MgCl_2$, 0.1 mM EDTA, 0.75 mM DTT, 7.5% glycerol, 0.05% NP-40, 3 μg BSA, 2 μg poly d(I-C) for 20 min in the presence of protease inhibitors (Marti et al., 1994*a*). Complexes were resolved on a 6% polyacrylamide gel using 0.25 × TBE as buffer.

Results

A Drop in the Level of Glucocorticoid Hormone Is Essential for Mouse Mammary Gland Involution and Programmed Cell Death of Epithelial Cells

Glucocorticoid hormones are essential for lobular and alveolar development during pregnancy and milk protein synthesis during lactation. We determined the level of glucocorticoid hormones at lactation and after 3 d of involution in the serum of circulating blood and found a significant drop from 34 to 14 nmol per liter ($p < 1\%$). We investigated whether an artificial elevation of glucocorticoid hormone levels is sufficient to maintain the lobulo-alveolar structure of the gland and to prevent PCD of secretory epithelial cells (Fig. 1 *a*). Deoxycorticosterone-releasing plastic pellets were implanted into the fourth inguinal mammary gland of a lactating animal and the pups were removed at the same time. The animals were killed 3 d later and the glands were analyzed histologically and with respect to DNA fragmentation by in situ terminal transferase assay. A partial preservation of the lobulo-alveolar structure was maintained in the close vicinity of the pellet and a normal alveolar regression and enlargement of adipocytes was observed more distantly to the implanted pellet (Fig. 1 *a*, *top*) or in the contralateral gland of the same animal (data not shown). A terminal transferase assay was performed from sections prepared from tissue close to the pellet. Fig. 1 *a* (*bottom*) shows that epithelial cells were predominantly negative around the pellet (*left*) whereas a majority of epithelial cells in alveoli located more distant to the pellet stained positive (*right*). These results imply that involution and DNA fragmentation is inhibited by a local release of glucocorticoid hormones and that the effect is independent of the involvement of systemic effects in the animal.

Progesterone Inhibits Involution and Programmed Cell Death in the Mammary Gland

Glucocorticoid hormones are important for the maintenance of milk protein synthesis during lactation. It is therefore possible that the inhibition of involution and PCD in the area of the glucocorticoid-release pellets is due to the maintenance of a terminally differentiated state of secretory epithelial cells rather than an active inhibition of the cell death process. To further define the mechanism by which glucocorticoids inhibit involution and PCD, the effect of additional steroid hormones on the involution process was studied. Progesterone exerts its action by binding to the progesterone receptor. It is mainly promoting proliferation during pregnancy and has no essential function on milk protein synthesis during lactation (Friesen and Cowden, 1989). Progesterone-release pellets were implanted into the mammary gland of lactating animals and the pups were removed. 3 d postoperation the implanted mammary glands were analyzed histologically and by in situ terminal transferase assay. Fig. 1 *b* demonstrates a partial preservation of the alveolar structure (*top*) and an absence of labeled nuclei after terminal transferase reaction in the vicinity of the pellet (*bottom, left*). More distantly, the gland was morphologically indistinguishable from a mammary gland during involution and most epithelial cells in these alveolar structures were positive in a terminal transferase reaction (*right*). These results demonstrate that progesterone and glucocorticoid similarly prevent involution and PCD in the vicinity of the pellets but not in areas more distant to the pellets.

Inhibition of Involution by Dexamethasone Is Systemic and Affects All Glands of the Animals

A similar series of experiments was performed with dexamethasone-, prolactin-, or as a control BSA-release pellets implanted into the fourth mammary gland after 5 d of lactation. The pups were removed and the glands were analyzed 3 d later. Dexamethasone efficiently inhibited a collapse of lobulo-alveolar structures (Fig. 2 *a*) and the alveoli were enlarged and filled with milk whereas BSA had no inhibitory effect (Fig. 2 *c*). At the same time no degradation of chromosomal DNA could be detected by in situ terminal transferase assays in dexamethasone-release pellet implanted glands (Fig. 2 *b*). In the case of BSA many epithelial cell nuclei stained positive indicating that DNA fragmentation has occurred (Fig. 2 *d*). The effect of dexamethasone was found to be systemic and to affect not only the gland implanted with the dexamethasone-release pellet but all the mammary glands of the animal (data not shown). Prolactin was found to be ineffective in preventing mammary gland involution and DNA degradation when released from a pellet (Fig. 2, *e* and *f*). A combination of dexamethasone and prolactin was equally efficient in preventing involution as dexamethasone alone (Fig. 2, *g* and *h*).

Taking advantage of the systemic effect exerted by dexamethasone additional molecular parameters of involution and PCD were investigated. Total DNA was isolated from the gland and analyzed by gel electrophoresis. As can be seen in Fig. 3 an oligonucleosomal fragmentation of DNA typical for cells undergoing programmed cell death was apparent in control glands implanted with a BSA-containing pellet (lane *2*) or a prolactin-containing pellet (lane *4*) while no DNA fragmentation was apparent in the DNA

Figure 2. Dexamethasone but not prolactin prevents involution and PCD. At lactation mammary glands were implanted with dexamethasone-release pellets (*a* and *b*), BSA-release pellets (*c* and *d*), prolactin-release pellets (*e* and *f*), or dexamethasone/prolactin-release pellets (*g* and *h*). The pups were removed and the glands were analyzed 3 d later. Shown are hematoxylin/eosin stained sections (*a, c, e,* and *g*) next to the pellet (*P*). The right panels (*b, d, f,* and *h*) show the result of a terminal transferase assay derived from serial sections of the same areas of the glands. The pellets (*P*) are visible at the left end of each panel. Bar, 100 μm.

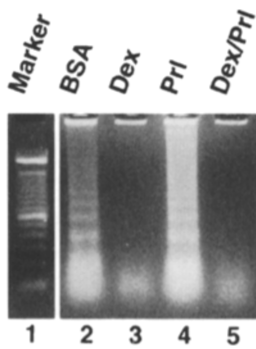

Figure 3. DNA fragmentation is inhibited by dexamethasone but not by prolactin. Mammary glands were implanted at lactation with BSA-release pellets (lane *2*), dexamethasone-release pellets (lane *3*), prolactin-release pellets (lane *4*), or dexamethasone/prolactin-release pellets (lane *5*). The pups were removed and the glands were analyzed 3 days later. Shown is the DNA after electrophoretic separation on a 1.5% agarose gel. A 100-bp DNA ladder is shown in lane *1*.

prepared from glands implanted with release pellets containing dexamethasone or dexamethasone/prolactin (lanes *3* and *5*, respectively).

We have previously shown a striking induction of nuclear PKA activity in the mammary gland in the early phase of involution that precedes and probably contributes to an elevated level of *fos* and *jun* gene expression and AP-1 DNA binding activity in the involuting gland. The level of PKA activity was determined in nuclear extracts prepared from mammary glands 2 and 3 d after implanting a dexamethasone-, prolactin-, dexamethasone/prolactin-, or control BSA-containing pellet. As can be seen from Fig. 4 *a* none of the release pellets was capable to inhibit an activation of nuclear PKA activity in these glands (lanes *4–11*) and the levels were similar to the levels regularly measured during normal involution (lanes *2* and *3*). It was of interest to investigate whether *fos* and *jun* gene expression and AP-1 DNA binding activity, which are putative targets of PKA in these cells, would be elevated in pellet-implanted glands. DNA binding activity was determined by bandshift analysis. AP-1 was found to be elevated in all pellet-implanted glands and the level of activity was found to be comparable to the level determined at day three of normal involution (Fig. 4 *b*). We also determined the DNA binding activity of the mammary gland factor MGF that regularly decreases during normal involution (Schmitt-Ney et al., 1992). In animals implanted with dexamethasone or prolactin-release pellets we found a high level of MGF DNA binding activity and the level was significantly lower in mammary glands of control animals or in animals implanted with a BSA control pellet (data not shown).

We also determined the level of expression of milk protein genes, and the putative AP-1–dependent sulfated glycoprotein gene (SGP-2), *fos* and *jun* genes and the metalloprotease stromelysin-1 gene, both, in animals implanted with dexamethasone-release pellets or BSA-release control pellets. The expression of WAP, a major milk protein, is maintained at a high level for 4 d in mammary glands implanted with a dexamethasone-release pellet and the level drops to a very low level in control animals implanted with a BSA-release pellet (Fig. 5). During the same period the level of expression of SGP-2 was increased to a lower level in glands implanted with dexamethasone-release pellets as compared to glands of control animals implanted with BSA-release pellets. Similar increases were observed in the expression of *jun*B and *jun*D in hormone-exposed and in control glands (Fig. 5). The level of c-*fos* measured in glands that were exposed to dexamethasone was elevated as compared to control glands (Fig. 5). The promoter of the *fos* gene is complex and contains multiple control elements that confer positive responsiveness towards elevated cAMP and PKA (Fisch et al., 1989; Boutillier et al., 1992) as well as negative responsiveness towards AP-1 (Schönthal et al., 1989). c-*jun* that is expressed during normal involution and is at least in part autoregulated by AP-1 (Angel et al., 1988) was induced to a lower level in glands implanted with dexamethasone-release pellets as compared to glands of control animals implanted with BSA-release pellets (Fig. 5). Most strikingly, we found a strong

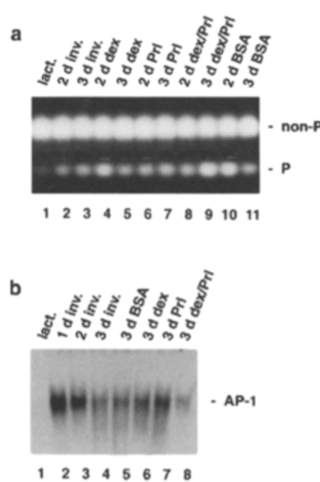

Figure 4. PKA and AP-1 are induced in pellet implanted mammary glands. Hormone-release pellets containing dexamethasone, prolactin, dexamethasone/prolactin, or BSA were implanted at lactation and the pups were removed. Nuclear extracts were prepared from unimplanted glands at lactation and after 1, 2, or 3 d of involution or from glands 2 or 3 d after implantation with hormone-release pellets as indicated. *a* shows the result of a PKA activity. Phosphorylated (*P*) and non-phosphorylated peptide (*non-P*) were separated electrophoretically. AP-1 DNA binding activity was determined by bandshift analysis (*b*). Only the area of the gel containing the protein/DNA complex is shown.

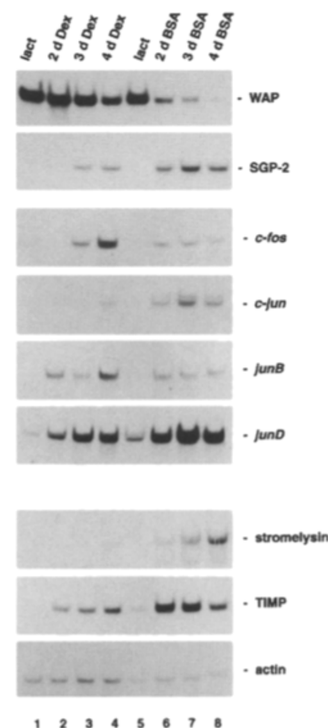

Figure 5. Dexamethasone inhibits the expression of AP-1–dependent genes. Hormone-release pellets containing dexamethasone (lanes *2–4*) or BSA (lanes *6–8*) were implanted at lactation and pups were removed. RNA was extracted from glands at lactation (lanes *1* and *5*) or from pellet-implanted glands after 2, 3, or 4 d and analyzed for the expression of WAP, SGP-2, c-*fos*, c-*jun*, *jun*B, *jun*D, stromelysin-1, TIMP-1, or actin by Northern blot analysis as indicated.

inhibition of AP-1–dependent stromelysin-1 gene expression in animals implanted with dexamethasone-release pellets but not in control animals suggesting that the AP-1 complex that accumulates in these dexamethasone-exposed glands is in a non-functional conformation. The expression of TIMP-1, a functional antagonist of metalloproteases, was only moderately affected by dexamethasone and actin expression was used as a control.

Discussion

We demonstrate that glucocorticoid hormones inhibit involution and PCD. Deoxycorticosterone acetate maintains the alveolar morphology and inhibits nuclear DNA fragmentation in the vicinity of the pellet but not distant to the pellet indicating that the response of the mammary tissue towards glucocorticoids is local and does not involve a systemic signal. Our results suggest that glucocorticoids that are important regulators of milk protein synthesis may in addition act as survival factors in mammary epithelial cells. An activity of additional survival factors such as EGF or EGF-like peptides or insulin-like peptides on epithelial cells in culture was recently demonstrated (Merlo et al., 1995) and further studies will be required to evaluate whether glucocorticoids affect the activity of any of these hormones in vivo. Interestingly, progesterone was similarly shown to inhibit involution and PCD in the vicinity of the pellet. This points to an inhibitory mechanism that is shared by different steroid receptors. Earlier studies have shown that daily injections of high doses of glucocorticoids inhibited mammary involution in vivo and in vitro (Johnson and Meites, 1958; Ossowksi et al., 1979). Prolactin had a similar effect but only when injected into the animal and not in mammary explants cultured in vitro (Ossowski et al., 1979). We show that prolactin fails to inhibit involution and PCD when administered locally. This supports the hypothesis postulated by Ossowski et al. (1979) that prolactin exerts its action indirectly by sensitizing the animal towards other hormones.

In contrast to deoxycorticosterone acetate, dexamethasone affects all mammary glands equally. This can probably be explained by the high stability of dexamethasone that leads to elevated hormone levels in the serum. In fact, the endogenous glucocorticoid hormone levels were strongly reduced in animals implanted with a dexamethasone-release pellet (<3 nMol per liter 4 d after implantation, data not shown), probably as a result of a suppression of the adrenal glands in these animals. Furthermore, a similar inhibition of involution was observed when a dexamethasone-release pellet was implanted under the skin distant from the mammary gland (data not shown). Several markers have been described previously that are upregulated during involution. Among them are SGP-2, TGF-β1, and stromelysin-1 (Strange et al., 1992), plasminogen activator (Ossowski et al., 1979), PKA, c-*fos*, c-*jun*, *jun*B, and *jun*D (Marti et al., 1994*a*). Our results demonstrate that relatively early events such as the induction of PKA and AP-1 DNA binding activity are not significantly altered by dexamethasone. However, the expression of SGP-2, c-*jun*, and stromelysin-1 mRNA is inhibited in the presence of dexamethasone (Fig. 5). Interestingly, these genes contain AP-1 response elements in their promoter regions and are potential target genes of AP-1 (Angel et al., 1988; McDonnell et al., 1990; Kerr et al., 1992; Wong et al., 1993, 1994; F. Martin, personal communication). TGF-β1 was also shown to be an AP-1–regulated gene (Kim et al., 1990). A partial inhibition of TGF-β1 mRNA induction was found in the mammary gland in the presence of dexamethasone (data not shown). Several steroid hormone receptors including those for glucocorticoids and progesterone have been shown to functionally interfere with AP-1 (Jonat et al., 1990; Lucibello et al., 1990; Yang-Yen et al., 1990; Shemshedini et al., 1991). In the case of the glucocorticoid-mediated AP-1 repression it was shown that the glucocorticoid receptor affects AP-1 function without disturbing the DNA binding capacity of AP-1 (Jonat et al., 1989; König et al., 1992). It is very likely that the inhibition of stromelysin-1, c-*jun*, SGP-2, and TGF-β1 gene expression is at least in part due to a functional inhibition of AP-1 in animals implanted with glucocorticoid-release pellets. Our results demonstrate that this inhibition is paralleled by an inhibition of involution and PCD in the mammary gland.

Interestingly, we found elevated c-*fos* mRNA levels in the presence of dexamethasone that could also be a consequence of glucocorticoid receptor-mediated AP-1 inactivation. The *fos* promoter is complex and it was shown to confer positive responsiveness to various growth factors (Greenberg and Ziff, 1984; Kruijer et al., 1984; Müller et al., 1984) and also to cAMP and PKA (Fisch et al., 1989; Boutillier et al., 1992). Furthermore, c-*fos* expression was shown to be regulated by AP-1 through a negative feedback mechanism (Schönthal et al., 1989; Gauthier-Rouvière et al., 1992). It is likely that the elevated PKA activity contributes to c-*fos* expression during involution. The relatively higher level of expression in dexamethasone-exposed glands as compared to control glands could be a consequence of an absence of AP-1–mediated down-regulation. This independently suggests that AP-1 function is impaired in glands exposed to dexamethasone.

The observed inhibition of stromelysin-1 expression could affect involution very significantly as it was demonstrated that the degradation of the basement membrane by metalloproteases is an important step of mammary gland involution (Talhouk et al., 1992) and promotes apoptosis of epithelial cells in culture (Boudreau et al., 1995). A function of AP-1 would therefore be to contribute to the basement membrane degradation during involution by stimulating the expression of metalloproteases such as stromelysin-1. This hypothesis was recently further substantiated by results based on in situ hybridizations where the expression of stromelysin-1 and SGP-2 was shown to take place in epithelial cells (Z. Feng, K. Guo, and R. Friis, personal communication). c-Fos and JunD, which are the major components of AP-1 during involution (Marti et al., 1994*a*) were recently shown to be expressed in the mammary epithelium during involution as measured by immunohistochemical studies (Marti et al., 1995). Furthermore, we have shown that an activation of PKA during lactation by the means of cholera toxin-release pellets leads to an activation of AP-1 and to PCD of epithelial cells in the vicinity of the pellet (Z. Feng, A. Marti, and R. Jaggi, manuscript in preparation).

Independent studies have shown increased levels of p53 expression during mammary gland involution (Strange et

al., 1992; Marti et al., 1995). We measured a partial inhibition of p53 expression at the mRNA level in dexamethasone-exposed glands as compared to control glands (data not shown). A putative role of p53 was discussed during involution of the mouse prostate, another hormone-dependent gland. Whereas Berges et al. (1993) claimed that prostate involution occurs normally in p53 knock out mice, Colombel et al. (1995) demonstrated that apoptosis and involution are delayed in the absence of p53. Preliminary studies in p53 knock out mice suggest that involution is delayed but not impaired in mammary glands of these mice (Feng, Z., R. Friis, and A. Clarke, unpublished observation). Cell death induced by serum-free medium in several mammary epithelial cell lines in culture was also shown to be independent of p53 (Merlo et al., 1995). These observations would suggest that PCD of mammary epithelial cells can occur in the absence of p53.

In summary, our data suggest that several steroid hormones are able to maintain the morphology of the lactating mammary gland and to inhibit mammary involution and PCD of mammary epithelial cells. To date, the only function that is known to be shared by different steroid hormone receptors is their capacity to functionally impair transcription factor AP-1. Based on the observation that the activation of PKA and the expression of genes that are regulated via PKA are not affected by steroid hormones implies that the steroid hormone-mediated effects are downstream of these initial events and most probably affect AP-1 at the level of its biological activity. The fact that the inhibition of AP-1 function is paralleled by an inhibition of involution and PCD of mammary epithelial cells suggests that AP-1 exerts an important function during mammary gland involution. Further studies are required to elucidate in detail the molecular consequences of AP-1 regulated genes on the actual process of PCD in dying mammary epithelial cells.

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