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Hormonal regulation of plasminogen activator in rat hepatoma cells

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Summary

Plasminogen activators are membrane-associated, arginine-specific serine proteases which convert the inactive plasma zymogen plasminogen to plasmin, an active, broad-spectrum serine protease. Plasmin, the major fibrinolytic enzyme in blood, also participates in a number of physiologic functions involving protein processing and tissue remodelling, and may play an important role in tumor invasion and metastasis. In HTC rat hepatoma cells in tissue culture, glucocorticoids rapidly decrease plasminogen activator (PA) activity. We have shown that this decrease is mediated by induction of a soluble inhibitor of PA activity rather than modulation of the amount of PA. The hormonally-induced inhibitor is a cellular product which specifically inhibits PA but not plasmin. We have isolated variant lines of HTC cells which are selectively resistant to the glucocorticoid inhibition of PA but retain other glucocorticoid responses. These variants lack the hormonally-induced inhibitor; PA from these variants is fully sensitive to inhibition by inhibitor from steroid-treated wild-type cells. Cyclic nucleotides dramatically stimulate PA activity in HTC cells in a time- and concentration-dependent manner. Paradoxically, glucocorticoids further enhance this stimulation. Thus glucocorticoids exert two separate and opposite effects on PA activity. The availability of glucocorticoid-resistant variant cell lines, together with the unique regulatory interactions of steroids and cyclic nucleotides, make HTC cells a useful experimental system in which to study the multihormonal regulation of plasminogen activator.

Introduction

Plasminogen activators (PAs) are membrane-associated arginine-specific serine proteases found in a variety of tissues (1). PA selectively hydrolyses a single Arg-Val bond of the plasma zymogen, plasminogen, to yield the active serine protease, plasmin, the major fibrinolytic activity in blood (2, Fig. 1). Plasmin is a broad-spectrum endopeptidase which can act on a variety of proteins. Because plasminogen is present in plasma in relatively high concentrations (1.5 to 2 μM, or 0.5% of all plasma proteins), the plasminogen activator-plasmin cascade provides considerable potential proteolytic activity (2, 3). Thus generation of plasmin both amplifies PA activity and broadens the substrate specificity. In addition to plasmin's well-known role in fibrinolysis, it is also involved in many normal physiologic functions which involve protein processing, cell migration and tissue remodelling (1, 3, 4, Table 1). By acting directly on fibrin and directly or indirectly (via activation of procollagenase) on connective tissue matrix (5, 6), the plasminogen activator-plasmin cascade may also play an important role in tumor invasion and metastasis (1, 3, 4, 6).

Not surprisingly for an enzyme of such biological importance, plasminogen activator is subject to regulation by a variety of effectors (see 7 for review). Steroid (8–16) and polypeptide hormones

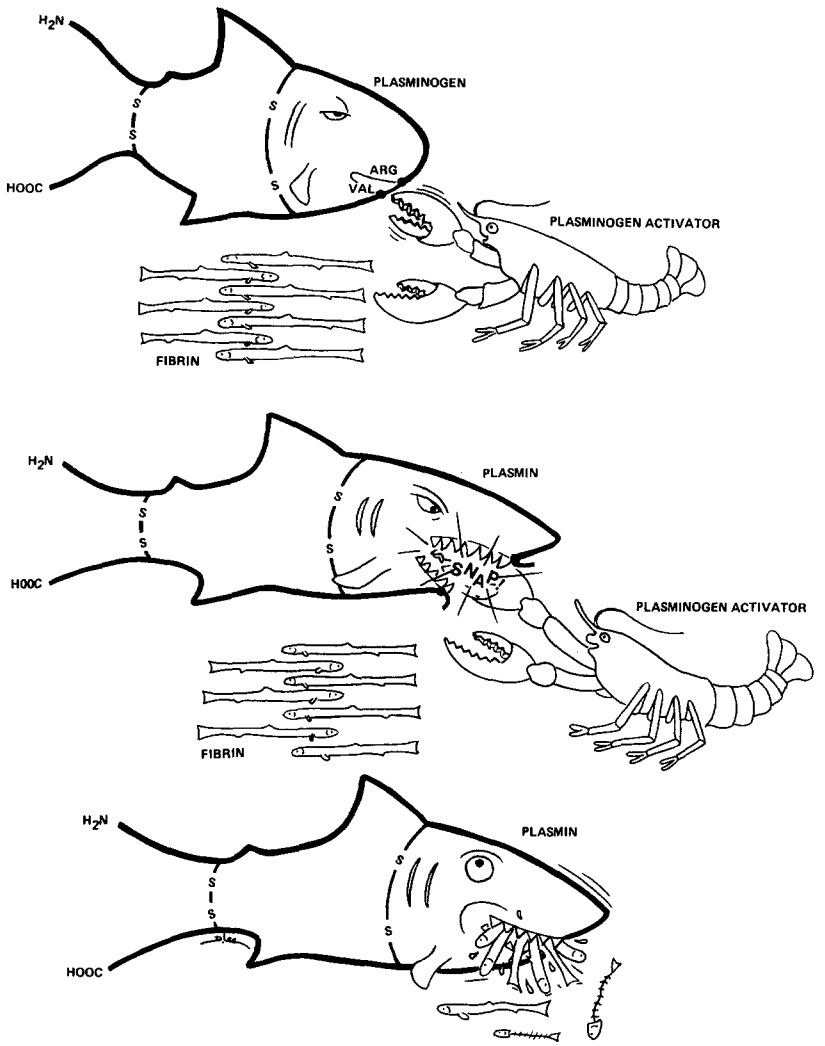

Fig. 1. Cartoon representation of plasminogen activation.

Table 1. Physiological/pathological processes mediated by plasminogen activator/plasmin.

Fibrinolysis
Proteolytic processing of cellular and serum proteins
Complement activation
Kinin formation
Proinsulin conversion
Migration of macrophages during inflammation
Tissue remodelling and destruction
Rupture of the ovarian follicle during ovulation
Implantation of the mouse embryo
Involution of the mammary gland following lactation
Neoplasia
Invasiveness
Metastatis

(17, 18), growth factors (4), cyclic nucleotides (13, 14, 17–22), retinoids (22–25), lectins (26) and tumor promoters (4, 22, 25, 27) are all modulators of PA activity in some tissues. Inhibitors of PA and/or plasmin are also important regulators of protease activities (2, 28–33), and in some experimental systems these inhibitors may also be subject to physiologic regulation (10, 11, 28, 33). The mechanisms by which PA activity is regulated are largely unknown, but could involve regulation of the rates of synthesis or degradation of the PA protein, activation of PA itself or of a PA precursor, or regulation of specific inhibitors of PA.

HTC cells are an established line of rat hepatoma

cells in long-term tissue culture, which provide a favorable experimental system for studying the regulation of plasminogen activators. This line is extremely well characterized with respect to hormonal regulation of multiple functions, particularly for the actions of glucocorticoids, insulin, and cyclic nucleotides (34–38). Furthermore, it is possible to isolate variant HTC cell lines altered in hormonal regulation of various properties, and several such lines have been described (9, 39, 40). Over the past several years we have exploited these features of HTC cells to study the hormonal regulation of plasminogen activator and the role of PA in various cellular functions modulated by hormones. We have described two unique mechanisms of regulation of PA: first, the glucocorticoid induction of a specific inhibitor of plasminogen activator (10, 11, 28); and second, a paradoxical effect of glucocorticoids on PA regulation in which glucocorticoids alone inhibit PA activity but together with cyclic nucleotides enhance the dramatic stimulation of PA activity by the latter (41).

Materials and methods

Cell culture

HTC cells were routinely grown in spinner or monolayer culture in Minimal Essential Medium (Eagle's) without antibiotics, modified to contain 50 mM tricine, a nonvolatile buffer, 0.5 g/l sodium bicarbonate, and supplemented with 2 mM glutamine and 5% calf and 5% fetal bovine serum. Experiments were performed in a chemically-defined medium identical to the growth medium except that it lacked serum and was supplemented with neomycin and, where applicable, with 0.1% bovine serum albumin.

Assays of plasminogen activator

PA was routinely assayed in either conditioned medium (CM) or 0.2% Triton X-100 extracts of cells using either an ^{125}I-fibrinolytic assay (42) or the esterolytic assay (43, 44) developed in this laboratory. HTC cells have no demonstrable plasminogen-independent fibrinolytic, caseinolytic, or esterolytic activity. Direct addition of dexamethasone or cyclic nucleotides to the assay mixture has no effect on PA activity. Inhibitory activity was measured by incubating CM or cell extracts to be tested with either CM or cell extracts of untreated HTC cells (as a source of PA) or urokinase (a human urinary plasminogen activator) for 20 min at 37 °C or 30 min at 25 °C prior to assaying PA activity. Inhibitory activity was quantitated by titrating serial dilutions of CM or cell extracts from dexamethasone-treated cells against a fixed amount of UK or HTC cell PA.

Characterization of plasminogen activator in HTC cells

Multiple molecular weight forms of PA were separated by SDS polyacrylamide gel electrophoresis under nonreducing conditions. Following electrophoresis, SDS was removed from the gels and proteins by exchange with the nonionic detergent Triton X-100, allowing recovery of PA activity (45). PA activity was then localized and assayed either by elution of PA from homogenized gel slices and assay of plasminogen-dependent fibrinolysis, by the fibrin-agar underlay method of Granelli-Piperno & Reich (45), or by plasminogen-dependent caseinolytic activity in the gel (46, 47).

Enucleation of HTC cells

Anucleate HTC cells (cytoplasts) were prepared by centrifugation of cells from suspension cultures through a discontinuous Ficoll gradient in the presence of cytochalasin B. The efficiency of enucleation was routinely greater than 92%. Cytoplast preparations maintained their membrane integrity for at least 24 hours in culture in the absence of serum (48).

Isolation of variant HTC cells

An agar-fibrin overlay technique (9, 49) was used to identify colonies with plasminogen-dependent fibrinolytic activity. Colonies which expressed PA activity in the presence of dexamethasone (and were thus presumably resistant to the dexamethasone inhibition of PA) were isolated through the agar-fibrin overlay and propagated in the absence of dexamethasone. Variant cell lines highly resistant to the dexamethasone inhibition of PA activity were obtained after several cycles of such treatment (9, 39).

Materials

Tissue culture media and sera were obtained from Gibco. Dexamethasone was the kind gift of

Dr Walter Gall of Merck Laboratories. 8-bromo-cAMP was purchased from Boehringer-Mannheim and Sigma, and the phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine, was obtained from Calbiochem-Behring. ^{125}I-fibrinogen was purchased from Amersham-Searle and Abbott Laboratories. Fibrinogen (97% clottable) was obtained from Calbiochem-Behring and used without further purification. Plasminogen was prepared from outdated human plasma by lysine-Sepharose chromatography (50). Urokinase was purchased from Collaborative Research or Calbiochem-Behring. Plasminogen was iodinated using the lactoperoxidase method (51).

Results

Glucocorticoid regulation of plasminogen activator

Plasminogen activator activity is found in the membrane fraction of HTC cells from which it can be released by detergents such as Triton X-100 (10). Activity is also found in serum-free medium conditioned by HTC cells (9, 41, 43). Analysis of PA activity from both cell extracts and conditioned medium on SDS polyacrylamide gels under nonreducing conditions reveals two major molecular weight forms of PA activity of 110 000 and 66 000 daltons. A minor band of activity is sometimes observed at 33 000 daltons (Fig. 2). There does not appear to be any difference between the molecular weights of the PAs associated with the cell and those released into the medium.

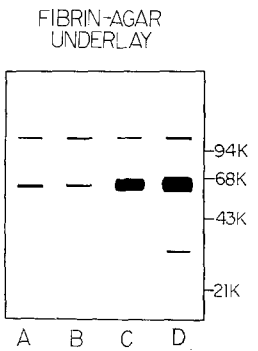

Fig. 2. Schematic diagram of multiple molecular weight forms of HTC cell plasminogen activator. Monolayer cultures of HTC cells were incubated for 24 hours in serum-free medium without hormones (Lane A), with 0.1 μM dexamethasone (Lane B), with 3 mM 8-bromo-cAMP/1 mM MIBX (Lane C), or with 3 mM 8-bromo-cAMP/1 mM MIBX/0.1 μM dexamethasone (Lane D). Conditioned media were subjected to SDS polyacrylamide gel electrophoresis and PA activity was localized on a fibrin-agar underlay as described in Materials and methods. Molecular weight markers are phosphorylase B (94K), bovine serum albumin (68K), ovalbumin (43K) and soybean trypsin inhibitor (21K).

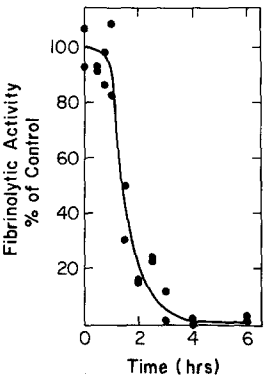

Fig. 3. Time course of dexamethasone inhibition of HTC cell plasminogen activator. HTC cells were incubated for the times indicated in serum-free medium containing 1 μM dexamethasone. Fibrinolytic activity of cell extracts was measured as described in Materials and methods and was normalized for the amount of protein in each sample. Each point represents the average of duplicate assays on a single culture. Reproduced from reference 10.

Glucocorticoids rapidly inhibit the activity of PA in HTC cells. Inhibition is half-maximal after approximately 90 min and maximal after 4 to 6 hours of incubation (Fig. 3). The magnitude of inhibition is usually 75 to 100%. Inhibition of PA activity is also observed in conditioned medium at later times than in cell extracts. Half-maximal inhibition is achieved at 5 nM dexamethasone, the same concentration that half-maximally induces tyrosine aminotransferase and half-maximally inhibits amino acid transport in HTC cells; maximal inhibition is achieved at 10 to 100 nM dexamethasone (39).

Dexamethasone could inhibit PA activity by decreasing the amount of PA protein or by decreasing its activity (possibly by inducing an inhibitor of this protease), or by some combination of these mechanisms. When increasing amounts of an extract of dexamethasone-treated cells are mixed with a fixed amount of an extract from control cells (as a source of PA activity), there is a concentration-dependent inhibition of PA activity, demonstrating that the dexamethasone-treated cells contain an inhibitor of

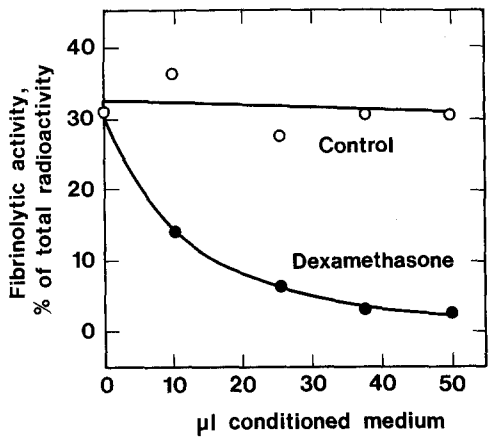

Fig. 4. Inhibition of HTC cell plasminogen activator by conditioned medium from dexamethasone-treated HTC cells. HTC cells were incubated for 18 hours with 0.1 μM dexamethasone (●) or without hormones (○). Increasing amounts of conditioned medium from these cultures were incubated for 30 min at 25 °C with 10 μg of cell extract from untreated HTC cells (as a source of PA) and fibrinolytic activity assayed as described. Each point represents the average of duplicate assays.

PA (10, 11). Cell fractionation experiments have demonstrated that the inhibitor is found primarily in the soluble 100 000 × **g** supernatant fraction (10), as well as in medium conditioned by dexamethasone-treated cells (Fig. 4).

An intact nucleus is required for this hormonal regulation of PA activity. Cytoplasts prepared by

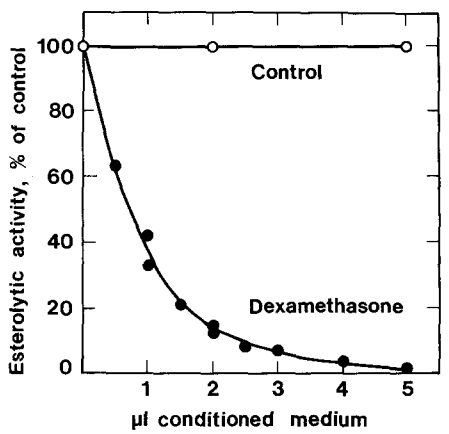

Fig. 5. Inhibition of human urokinase by conditioned medium from dexamethasone-treated HTC cells. HTC cells were incubated for 18 hours with 0.1 μM dexamethasone (●) or without hormones (○). Increasing amounts of conditioned medium were incubated for 20 min at 37 °C with 5 milliPloug units of human urokinase and esterolytic activity assayed as described (44). Each point represents the average of duplicate assays.

centrifugation through Ficoll gradients in the presence of cytochalasin B maintain PA activity at levels comparable to or higher than those of intact cells. However, anucleate HTC cells are not responsive to glucocorticoid regulation of PA activity. Dexamethasone does not decrease either intracellular or extracellular PA activity of the anucleate cells, or induce production of the soluble inhibitor of PA activity (52).

The dexamethasone-induced inhibitor of PA activity (PAI) also inhibits the plasminogen-dependent fibrinolytic or esterolytic activity of HeLa cells (Coleman, unpublished work) and of human urokinase (Fig. 5), as well as PA from HTC cells. Thus, PAI inhibits both major immunochemical types of PA, urokinase-like and tissue activator (1). In contrast, plasmin is not inhibited by conditioned medium from HTC cells incubated with dexamethasone. The specificity of the inhibitor for plasminogen activation was demonstrated directly by the inhibition of urokinase-catalyzed activation of ^{125}I-plasminogen to ^{125}I-plasmin (28). These results show that the inhibition is not directed against plasmin, but is specific for plasminogen activator.

Because certain cell types can take up serum protease inhibitors from the serum in medium (31, 33, 53) and release them to serum-free medium conditioned by these cells (31), we investigated the origin of this hormonally-induced inhibitor in HTC cells. SF HTC-H1, a line of cells selected for their ability to grow in serum-free medium (54), were grown for 76 days (at least 30 generations) in the presence or absence of serum; dexamethasone induced equivalent amounts of inhibitory activity in cells grown under either condition. Furthermore, the inhibitory activity from HTC cells is stable to pH 3 for 2 hours at 37 °C, a treatment which inactivates fibrinolytic inhibitors in serum. These results indicate that the dexamethasone-induced inhibitor is a cellular product which differs from serum-derived fibrinolytic inhibitors (28).

The inhibitor is inactivated by boiling and by treatment with pepsin under acidic conditions, suggesting that it is a protein. The PA inhibitory activity in CM from dexamethasone-treated cells migrates as a single band of approximately 45 000 daltons upon SDS polyacrylamide gel electrophoresis under nonreducing conditions (Cwikel & Gelehrter, unpublished work). PAI is clearly different from the 38 000 dalton protease inhibitor, protease

nexin, described by Low et al. (32) which inactivates both thrombin and urokinase. Conditioned medium from dexamethasone-treated cells, which readily inhibited urokinase, had no effect on thrombin activity, even in the presence of heparin which accelerates the thrombin-protease nexin interaction (Coleman & Gelehrter, unpublished work).

We have begun to investigate the interaction of PAI with various PAs. Preliminary evidence suggests that PAI forms an irreversible covalent complex with urokinase. In contrast, its interaction with PA from HTC cells can be reversed under conditions of SDS polyacrylamide gel electrophoresis. Taking advantage of the latter fact, we have asked whether dexamethasone decreases PA activity directly, in addition to the effects mediated by PAI. Cell extracts and CM from dexamethasone-treated cells, which have less than 10% of control PA activity when assayed by the fibrin plate assay, exhibit PA activity comparable to the controls when measured by the fibrin-agar underlay (Fig. 2) or by direct assay of gel eluates following SDS polyacrylamide gel electrophoresis. These results suggest that PA and PAI dissociate during electrophoresis, and that there is no decrease in the amount of PA in cell extracts or medium from dexamethasone-treated cells (Cwikel, Coleman, Barouski-Miller & Gelehrter, unpublished work).

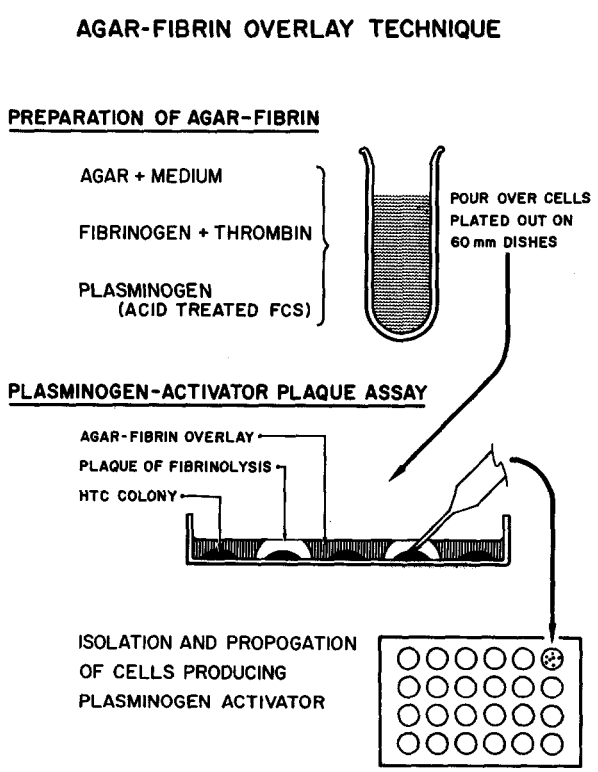

Figure 6. Schematic diagram of the agar-fibrin overlay technique used to isolate variant HTC cell lines resistant to the glucocorticoid inhibition of PA. Experimental details in Materials and methods and in references 9 and 39. Reproduced from reference 9.

Isolation and characterization of variant hepatoma cells resistant to hormonal regulation of plasminogen activator

Utilizing the agar-fibrin overlay technique (Fig. 6), we have isolated a number of variant HTC cell lines which are highly resistant to the dexamethasone inhibition of PA activity (9, 39). These variants are resistant to a concentration of dexamethasone 1 000 times greater than that necessary to completely inhibit PA activity in wild-type cells (39). The nature of the defect in these cells was defined by mixing experiments analogous to those described above. Dexamethasone-treated variant cells show no PAI activity, whereas the PA from these cells is fully sensitive to inhibitor from glucocorticoid-treated wild-type cells. Thus the basis of hormone resistance in the variants appears to be the failure of dexamethasone to induce PAI (10, 11).

The growth rate and cloning efficiency of variant and wild-type lines, both on plastic and in soft agar, are indistinguishable. Morphologically there are no consistent differences between the variant and wild-type cells. Fluctuation analyses support the hypothesis that resistance to dexamethasone arises randomly and is not induced by the hormone. It was not possible to determine the rate at which stable variant cells arise, but only to quantitate the frequency of the first step in this process. The frequency with which colonies from a wild-type population form fibrinolytic plaques in the presence of dexamethasone is high (approximately 10^{-3}) and this rate is not altered by treatment with two different mutagens: ethylmethane sulfonate and UV light. This observation suggests that mutations are not the primary cause of resistance in this cell line. The karyotypic variability of HTC cells raises the possibility that variants might arise from chromosomal segregation events (39).

Biochemical analysis of the variant cell lines

demonstrates that they have a lesion specific for the regulation of plasminogen activator. The hormonal resistance is apparently not due to deficient or defective steroid receptor function since the variants show wild-type induction of tyrosine aminotransferase. The lesion in these variants must therefore be at some step distal to the entry of the hormone-receptor complex into the nucleus. We have also shown that 6 of 7 variant cell lines tested show wild-type inhibition of amino acid transport by glucocorticoids (one variant is partially resistant to the inhibitory effect of dexamethasone on transport). These findings indicate that there is not a generalized resistance to all membrane-associated dexamethasone responses, but that the cells are selectively resistant to the inhibition of PA (39). Other variant HTC cell lines which are selectively resistant to the dexamethasone induction of tyrosine aminotransferase (40) show wild-type inhibition of PA activity by glucocorticoids (55). These results indicate that the various glucocorticoid-mediated responses in HTC cells are independently rather than coordinately regulated (11, 55). The selective resistance of these HTC variants is unique and in contrast to the great majority of glucocorticoid-resistant variant cell lines previously described, essentially all of which have been shown to have deficient or defective glucocorticoid receptors (56, 57).

We utilized these variant lines to study the role of plasminogen activator in the hormonal regulation of other membrane properties. HTC cells exhibit increased levels of adhesion to a substrate as well as decreased PA activity when incubated with dexamethasone (58). In a variety of cultured cells, adhesion to a substrate requires specific cell surface glycoproteins and intact cytoskeletal elements, and there is evidence that plasmin may affect both of these components of adhesion (59–61). Using the variant HTC cell lines, we tested the hypothesis that dexamethasone induces cell adhesion by decreasing the activity of PA, which in turn allows the accumulation of specific cell surface glycoproteins necessary for adhesion. If this hypothesis were correct there should be little or no dexamethasone inhibition of adhesion in the variant cell lines. A sensitive quantitative assay was developed which measures the strength of attachment of radioactively-labeled cells to glass scintillation vials following exposure to shearing force (62). We found that dexamethasone induces the adhesiveness of variant HTC cells to the same extent as that of wild-type cells. This was true when adhesion was measured in serum-free medium, in serum-containing medium, or in serum-containing medium depleted of, or reconstituted with, plasminogen. These results indicated that neither PA itself nor plasmin plays a major role in cell adhesion in HTC cells and suggested that the dexamethasone induction of adhesion might operate through the synthesis of cell surface glycoproteins (11, 62).

Cyclic nucleotide regulation of plasminogen activator activity

Incubation of HTC cells with cAMP derivatives stimulates cell-associated PA activity 8- to 20-fold and extracellular PA activity 30- to 1 300-fold. This time- and concentration-dependent increase is enhanced by phosphodiesterase inhibitors such as 1-methyl-3-isobutylxanthine (MIBX). Maximal stimulation of PA activity is observed at 3 mM 8-bromo-cAMP and half-maximal stimulation at 0.2 mM. A similar concentration dependence is noted with dibutyryl-cAMP. N^6-monobutyryl-cAMP also stimulated PA activity but cAMP itself did not; dibutyryl-cGMP inhibited PA activity. Increases in PA activity in cells incubated with 8-bromo-cAMP are first detectable at 4 hours in cell extracts and 6 hours in extracellular medium, and are maximal by 8 and 12 hours, respectively (Fig. 7). MIBX increases the level of maximal stimulation, but does not significantly alter the time course (41).

As noted above, dexamethasone decreases PA activity by induction of an inhibitor. Paradoxically, dexamethasone added simultaneously with cAMP derivatives causes a further 4-fold enhancement of the cyclic nucleotide stimulation of PA activity (41). Analysis of the molecular weight forms of PA under these conditions indicates that the same 110 K and 66 K forms are present in cells treated with cyclic nucleotides or cyclic nucleotides plus dexamethasone as in control and dexamethasone-treated cells. In addition a minor 33 K dalton form of PA is sometimes found in cells incubated with cAMP derivatives plus or minus dexamethasone (Fig. 2). Dexamethasone also profoundly alters the time course of the cyclic nucleotide enhancement of PA activity: increased activity is

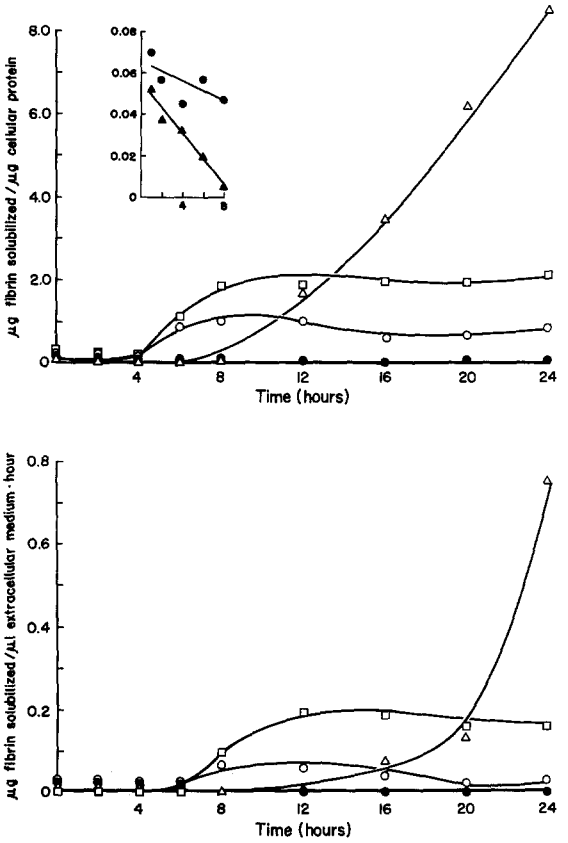

Fig. 7. Time course of 8-bromo-cAMP action in the presence and absence of dexamethasone. HTC cells were incubated for 0–24 hours in serum-free medium containing 0.1 μM dexamethasone (▲), 3 mM 8-bromo-cAMP (○), 3 mM 8-bromo-cAMP/1 mM MIBX (□), or 3 mM 8-bromo-cAMP/1 mM MIBX/0.1 μM dexamethasone (△). (●), Control (no additions). Upper panel: cell-associated PA activity. Lower panel: extracellular PA activity. Inset: expanded ordinate scale to demonstrate the effect of dexamethasone. In these experiments, the fibrinolytic activities of several different amounts of each sample were measured and the slope of the line (percentage of fibrin solubilized per microgram cellular protein or microliter of extracellular medium) was determined by linear regression analysis. PA activity is expressed as micrograms fibrin solubilized per microgram of cellular protein or microliter of extracellular medium. Reproduced from reference 41.

detected at 4 hours in cells incubated with 8-bromo-cAMP and MIBX but not until 12 hours in cells incubated with dexamethasone as well (41). Induction of inhibitor by dexamethasone might explain this delay in appearance of the cyclic nucleotide-stimulated increase in PA activity. Glucocorticoids thus exert two separate and opposite effects on PA activity: induction of an inhibitor and amplification of cyclic nucleotide action. Although permissive and synergistic effects of dexamethasone on cyclic nucleotide action have been reported previously (38, 63), glucocorticoid regulation of PA activity is unique in that the amplification of cyclic nucleotide effects by dexamethasone opposes its regulatory action toward a specific enzyme.

In variant HTC cells selectively resistant to the glucocorticoid inhibition of PA activity, dexamethasone still enhances the stimulation of PA activity by cyclic nucleotides (Fig. 8). Furthermore, in contrast to its effect in wild-type cells, dexamethasone does not alter the time course of 8-bromo-cAMP stimulation of PA activity in variant cells; enhancement is first observed at 6 hours and is maximal by 12 hours incubation. These results appear to dissociate the glucocorticoid induction of inhibitor from its enhancement of cyclic nucleotide stimulation of PA activity.

The steroid specificity of the glucocorticoid enhancement effect appears to be similar to that for glucocorticoid inhibition of PA activity and amino

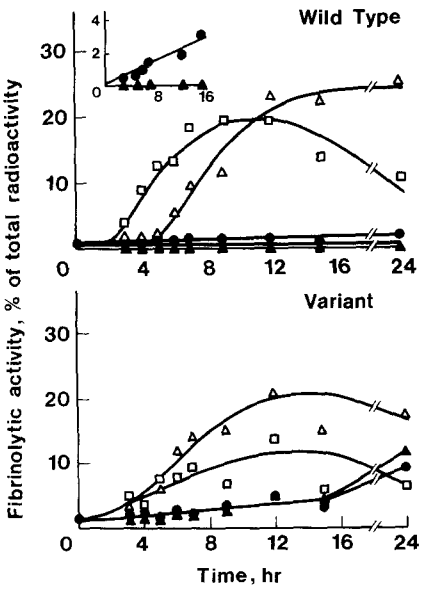

Fig. 8. Time course of 8-bromo-cAMP stimulation of PA activity in the presence or absence of dexamethasone in wild-type and variant cells. Wild-type and variant HTC cells were incubated for 0–24 hours in serum-free medium containing: no additions (●), 0.1 μM dexamethasone (▲), 3 mM 8-bromo-cAMP/1 mM MIBX (□), or 0.1 μM dexamethasone/3 mM 8-bromo-cAMP/1 mM MIBX (△). Cell-associated PA activity (in 2.5 micrograms cellular protein of wild-type cells or 1 microgram cellular protein of variant cells) was measured on ^{125}I-fibrin plates as described in Materials and methods, and expressed as a percentage of total radioactivity released.

acid transport (64), as well as induction of tyrosine aminotransferase (65). The optimal inducers, or full agonists, dexamethasone and cortisol, show a similar concentration dependence curve for all of these phenomena. In each case, dexamethasone was ten times more potent than cortisol. The partial agonists, 11β-hydroxyprogesterone and deoxycorticosterone, cause submaximal enhancement of the cyclic nucleotide stimulation of PA activity and do so only at higher steroid concentrations. Tetrahydrocortisol, which does not interact with the glucocorticoid receptor, fails to enhance cyclic nucleotide stimulation of PA activity. The glucocorticoid antagonist, 17α-methyltestosterone, which has no effect on the enhancement of cyclic nucleotide stimulation by itself, blocks the enhancement by dexamethasone in a concentration-dependent manner. These observations suggest that the steroidal enhancement of cyclic nucleotide stimulation of PA activity is mediated by the same glucocorticoid receptor mechanism which mediates the induction of transaminase, and the inhibition of amino acid transport and PA activity (7, Barouski-Miller & Gelehrter, unpublished work).

Incubation of HTC cells with cAMP derivatives also alters cell morphology, causing cell elongation and extension of processes followed by flattening of the cells. Plasminogen activator has been reported to alter cell morphology in several lines either directly (66) or by production of plasmin (67). We have shown, however, that the cyclic nucleotide effects on cell morphology are not caused by the stimulation of PA activity and can be dissociated from them. The morphologic changes appear within 30 to 60 min incubation with cyclic AMP derivatives, long before any detectable changes in either intracellular or extracellular PA are apparent. Upon removal of cyclic nucleotides from the medium, cell morphology returns to normal within two to four hours, a time at which PA activity is still significantly elevated. Furthermore, when protein synthesis is blocked by cycloheximide, the cyclic nucleotide stimulation of PA activity is completely blocked; however, induction of morphologic changes still occurs. Analogous to the situation described above for glucocorticoid regulation of PA and cell adhesion, these results suggest independent regulation by cyclic nucleotides of PA activity and cell morphology (7, Barouski-Miller & Gelehrter, unpublished work).

Discussion

HTC cells provide a useful experimental model for studying the hormonal regulation of plasminogen activator and the role of PA in several cellular functions modulated by hormones. In addition to various well-characterized hormonal responses in these cells, variant cell lines which are resistant to specific hormone-mediated functions have been isolated (9, 39, 40, 55, 68). Our studies have revealed two unique regulatory mechanisms: glucocorticoids inhibit PA activity by inducing a soluble inhibitor rather than by regulating the amount of enzyme (10, 11, 28); and glucocorticoids together with cyclic nucleotides paradoxically enhance the dramatic stimulation of PA activity by cyclic nucleotides (41).

Further investigation of this system should yield interesting information about mechanisms of hormonal regulation of this important protease. The ability to study the multiple molecular weight forms of PA should allow studies on the hormonal regulation of the expression of these forms. We can investigate whether various hormones cause differential regulation of these forms of plasminogen activator and whether they affect interconversion of these forms. The isolation and characterization of the dexamethasone-induced inhibitor (PAI) and the preparation of specific antibodies to it should allow studies on the hormonal regulation of PAI at a molecular level. Finally, the paradoxical effects of glucocorticoids on PA in this system provide a unique opportunity to study the nature of glucocorticoid-cyclic nucleotide interactions.

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References

1. Christman, J. K., Silverstein, S. C. and Acs, G., 1977. In: Proteinases in Mammalian Cells and Tissues. (Barrett, A. J., ed.), pp. 91–149, New York: North Holland Publishing Co.

2. Lijnen, H. R. and Collen, D., 1982. Seminars in Thrombosis and Hemostasis 8: 2–10.
3. Reich, E., 1978. In: Biological Markers of Neoplasia: Basic and Applied Aspects. (Ruddon, R. W., Jr., ed.), pp. 491–500, New York: Elsevier-North Holland.
4. Weinstein, I. B., Wigler, M., Yamasaki, H. et al., 1978. In: Biological Markers of Neoplasia: Basic and Applied Aspects. (Ruddon, R. W., Jr., ed.), pp. 451–471, New York: Elsevier-North Holland.
5. Werb, Z., Mainardi, C. L.,,Vater, C. A. and Harris, E. D., Jr., 1977. N. Engl. J. Med. 296: 1017–1023.
6. Quigley, J. P., 1979. In: Surfaces of Normal and Malignant Cells. (Hynes, R. O., ed.), pp. 247–285, Chichester: John Wiley.
7. Miller, P. A. Barouski-, 1982. Ph.D. thesis, University of Michigan.
8. Wigler, M., Ford, J. P. and Weinstein, I. B., 1975. In: Proteases and Biological Control. (Reich, E., Rifkin, D. B. and Shaw, E., eds.), pp. 849–856, New York: Cold Spring Harbor Laboratory.
9. Carlson, S. A. and Gelehrter, T. D., 1977. J. Supramolecular Structure 6: 325–331.
10. Seifert, S. C. and Gelehrter, T. D., 1978. Proc. Natl. Acad. Sci. U.S.A. 75: 6130–6133.
11. Gelehrter, T. D., Seifert, S. C. and Fredin, B. L., 1979. Cold Spring Harbor Conf. Cell Prolif. 6: 259–267.
12. Laishes, B. A., Roberts, E. and Burrowes, C., 1976. Biochem. Biophys. Res. Commun. 72: 462–471.
13. Vassali, J.-D., Hamilton, J. and Reich, E., 1976. Cell 8: 271–281.
14. Granelli-Piperno, A., Vassalli, J.-D. and Reich, E., 1977. J. Exp. Med. 146: 1693–1706.
15. Roblin, R. and Young, P. L., 1980. Cancer Research 40: 2706–2713.
16. Werb, Z., 1978. J. Exp. Med. 147: 1695–1712.
17. Beers, W. H., Strickland, S. and Reich, E., 1975. Cell 6: 387–394.
18. LaCroix, M. and Fritz, I. B., 1982. Molec. and Cell. Endocrinol. 26: 247–258.
19. Laug, W. E., Jones, P. A., Nye, C. A. and Benedict, W. F., 1976. Biochem. Biophys. Res. Commun. 68: 114–119.
20. Rosen, N., Piscitello, J., Schneck, J. et al., 1979. J. Cell. Physiol. 98: 125–136.
21. Rosen, N., Schneck, J., Bloom, B. R. and Rosen, O. M., 1978. J. Cyclic Nucleotide Research 5: 345–358.
22. Wilson, E. L. and Reich, E., 1979. Cancer Research 39: 1579–1586.
23. Strickland, S. and Mahdavi, V., 1978. Cell 15: 393–403.
24. Schroder, E. W., Chou, I.-N. and Black, P. H., 1980. Cancer Research 40: 3089–3094.
25. Miskin, R., Easton, T. G. and Reich, E., 1978. Cell 15: 1301–1312.
26. Mochan, E., 1979. Biochim. Biophys. Acta 558: 273–278.
27. Wigler, M. and Weinstein, I. B., 1976. Nature 259: 232–233.
28. Coleman, P. L., Barouski, P. A. and Gelehrter, T. D., 1982. J. Biol. Chem. 257: 4260–4267.
29. Loskutoff, D. J. and Edgington, T. S., 1977. Proc. Natl. Acad. Sci. U.S.A. 74: 3903–3907.
30. Roblin, R. O., Young, P. L. and Bell, T. E., 1978. Biochem. Biophys. Res. Commun. 82: 165–172.
31. Rohrlich, S. T. and Rifkin, D. B., 1981. J. Cell. Physiol. 109: 1–15.
32. Low, D. A., Baker, J. B., Koonce, W. C. and Cunningham, D. D., 1981. Proc. Natl. Acad. Sci. U.S.A. 78: 2340–2344.
33. Finlay, T. H., Katz, J., Rasums, A. et al., 1981. Endocrinology 108: 2129–2136.
34. Thompson, E. B., 1979. In: Glucocorticoid Hormone Action. (Baxter, J. D. and Rousseau, G. G., eds.), pp. 203–217, Heidelberg: Springer-Verlag.
35. Higgins, S. J., Baxter, J. D. and Rousseau, G. G., 1979. In: Glucocorticoid Hormone Action. (Baxter, J. D. and Rousseau, G. G., eds.), pp. 135–160, Heidelberg: Springer-Verlag.
36. Gelehrter, T. D., 1979. In: Glucocorticoid Hormone Action. (Baxter, J. D. and Rousseau, G. G., eds.), pp. 561–574, Heidelberg: Springer-Verlag.
37. Gelehrter, T. D., 1979. In: Glucocorticoid Hormone Action. (Baxter, J. D. and Rousseau, G. G., eds.), pp. 583–591, Heidelberg: Springer-Verlag.
38. Granner, D. K., 1979. In: Glucocorticoid Hormone Action. (Baxter, J. D. and Rousseau, G. G., eds.), pp. 593–611, Heidelberg: Springer-Verlag.
39. Seifert, S. C. and Gelehrter, T. D., 1979. J. Cell. Physiol. 99: 333–342.
40. Thompson, E. B., Aviv, D. and Lippman, M. E., 1977. Endocrinology 100: 406–419.
41. Barouski-Miller, P. A. and Gelehrter, T. D., 1982. Proc. Natl. Acad. Sci. U.S.A. 79: 2319–2322.
42. Strickland, S. and Beers, W. H., 1976. J. Biol. Chem. 251: 5694–5702.
43. Coleman, P. L. and Green, G. D. J., 1981. Annals, N.Y. Acad. Sci. 370: 617–626.
44. Coleman, P. L. and Green, G. D. J., 1981. Meth. Enzymol. 80: 408–414.
45. Granelli-Piperno, A. and Reich, E., 1978. J. Exp. Med. 148: 223–234.
46. Huessen, C. and Dowdle, E. B., 1980. Anal. Biochem. 102: 196–202.
47. Miskin, R. and Soreq, H., 1982. Anal. Biochem. 118: 252–258.
48. McDonald, R. A. and Gelehrter, T. D., 1981. J. Cell Biol. 88: 536–542.
49. Jones, P., Benedict, W., Strickland, S. et al., 1975. Cell 5: 323–329.
50. Deutsch, D. G. and Mertz, E. T., 1970. Science 170: 1095–1096.
51. Gilbert, L. R. and Wachsman, J. T., 1976. Anal. Biochem. 72: 480–484.
52. Barouski, P. A. and Gelehrter, T. D., 1980. Biochem. Biophys. Res. Commun. 96: 1540–1546.
53. Van Leuven, F., Cassiman, J-J. and Van den Berghe, H., 1979. J. Biol. Chem. 254: 5155–5160.
54. Thompson, E. B., Anderson, C. U. and Lippman, M. E., 1975. J. Cell. Physiol. 86: 403–412.
55. Thompson, E. B., Granner, D. K., Gelehrter, T. D. et al., 1979. Molec. and Cell. Endocrinol. 15: 135–150.
56. Pfahl, M. R., Kelleher, R. J. and Bourgeois, S., 1978. Molec. and Cell. Endocrinol. 10: 193–207.
57. Yamamoto, K. R., Gehring, U., Stampfer, M. R. and Sibley, C. H., 1976. Rec. Prog. Hormone Res. 32: 3–23.

58. Ballard, P. and Tomkins, G. M., 1970. J. Cell Biol. 47: 222–234.
59. Culp, L., 1978. Curr. Top. Membr. Transp. 11: 327–395.
60. Pollack, R. and Rifkin, D., 1975. Cell 6: 495–506.
61. Weber, J. M., 1975. Cell 5: 253–261.
62. Fredin, B. L., Seifert, S. C. and Gelehrter, T. D., 1979. Nature 277: 312–313.
63. Rousseau, G. G., 1977. Eur. J. Biochem. 76: 309–316.
64. Gelehrter, T. D. and McDonald, R. A., 1981. Endocrinology 109: 476–482.
65. Samuels, H. H. and Tomkins, G. M., 1970. J. Mol. Biol. 52: 57–74.
66. Quigley, J. P., 1979. Cell 17: 131–141.
67. Urquhart, C., Whur, P., Gordon, M. et al., 1978. Exptl. Cell Res. 113: 31–38.
68. Grove, J. R. and Ringold, G. M., 1981. Proc. Natl. Acad. Sci. U.S.A. 78: 4349–4353.

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