SHORT COMMUNICATION

Dexamethasone decreases urokinase plasminogen activator mRNA stability in MAT 13762 rat mammary carcinoma cells

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Summary The glucocorticoid dexamethasone was observed to decrease urokinase plasminogen activator (uPA) RNA levels from within 1 h of treatment of MAT 13762 mammary adenocarcinoma cells. The drug did not alter the rate of uPA gene transcription in these cells, but decreased the stability of cytoplasmic uPA mRNA transcripts. Results from cycloheximide and actinomycin D experiments indicated that the dexamethasone-mediated reduction in uPA RNA required both new protein and RNA synthesis. Based on these results, we propose that dexamethasone induces a short-lived protein(s) which down-regulates uPA RNA levels post-transcriptionally in these metastatic tumour cells.

Urokinase plasminogen activator (uPA) is a serine protease capable of cell-surface attachment and widely implicated as an initiator of tumour cell invasion during the metastatic process (Dano et al., 1985; Blasi et al., 1987). Expression of the uPA gene is known to be regulated transcriptionally (see North et al., 1990 and references therein), and also post-transcriptionally in the cytoplasm (Ziegler et al., 1990; Altius & Nagamine, 1991) and in the nucleus (Henderson et al., 1992a,b). The synthetic glucocorticoid, dexamethasone, exerts a negative influence on uPA mRNA levels in human tumour cell-lines (Busso et al., 1986; Busso et al., 1987; Medcalf et al., 1988), at a level which includes decreased transcription (Medcalf et al., 1988). We observed a similar effect of dexamethasone during our studies on uPA gene regulation in MAT 13762 rat mammary carcinoma cells, which normally express high levels of uPA mRNA and protein (Henderson & Kefferd, 1991; Henderson et al., 1992a). In this communication, we report an investigation of the mechanism(s) by which dexamethasone decreases uPA mRNA levels in these metastatic tumour cells.

Dexamethasone decreases uPA RNA levels

Densitometric quantitation of Northern blots revealed that 10^-7 M dexamethasone reduced uPA RNA levels from within 1 h, and resulted in a 4-fold decrease by 20 h (see Figure 1a). The glucocorticoid was equally effective at concentrations of 10^-8 to 10^-6 M (data not shown). Cycloheximide (CHX) inhibits protein synthesis in MAT 13762 cells by >95% (data not shown), and increased uPA RNA levels approximately 2-fold in 4 h in these cells (Figure 1b). Pretreatment of MAT 13762 cells with 10 μg ml^-1 cycloheximide completely blocked the effect of dexamethasone on uPA RNA levels (Figure 1b), as did addition of the transcription inhibitor, actinomycin D (Figure 1c). These results suggest that dexamethasone requires the continued synthesis of RNA and protein molecules to reduce uPA RNA levels in MAT 13762 cells. In addition, pretreatment with 10-fold molar excess of the glucocorticoid antagonist RU38486 blocked the hormone effect (data not shown), suggesting that dexamethasone is acting via the glucocorticoid receptor.

In contrast to the situation reported for human cell-lines (Busso et al., 1987), dexamethasone did not repress uPA gene transcription in MAT 13762 cells. RNA blot analysis and a nuclear run-on assay showed that whilst dexamethasone decreased both cytoplasmic and mature nuclear uPA RNA pools (Figure 2a), the rate of uPA gene transcription was unaltered (Figure 2b). The exact mechanism by which the nuclear uPA RNA level (which is unusually high relative to the cytoplasmic pool in MAT 13762 cells) is decreased by the drug is at present unclear, however the effect was gene-specific relative to a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) reference gene (Figure 2a).

Dexamethasone acts at a post-transcriptional level

The half-life of uPA RNA was estimated to be >24 h by actinomycin D chase (Figure 2c), suggesting that the rapid decrease observed in uPA RNA might result from an effect of dexamethasone on uPA cytoplasmic RNA stability. This was confirmed by a 3H-uridine pulse-chase assay (Figure 2d); a technique which avoids the need to use transcription inhibiting drugs, and which revealed that dexamethasone decreased the uPA RNA half-life to about 5.5 h. This is a novel finding with regard to uPA gene regulation. It should be pointed out however, that similar studies of uPA regulation by dexamethasone in human breast cancer cell-lines also reported a long half-life of uPA mRNA prior to drug treatment (Busso et al., 1986), suggesting that hormonal modulation of cytoplasmic uPA mRNA stability may be a more general phenomenon.

Dexamethasone is known to decrease the stability of other mRNAs including those encoded by the genes for c-myc (Maroder et al., 1990), interleukin-1β (Lee et al., 1988), interferon-β (Peppel et al., 1991) and the monococyte chemo-tactic-activating factor (MCAF) gene (Mukaida et al., 1991). A structural feature common to all of the transcripts encoded by these genes, and in particular the uPA genes (Henderson & Kefferd, 1991), is the presence of 3'-untranslated region A+U rich sequences, which signal degradation of cytokine and certain oncogene mRNAs (Shaw & Kamen, 1986; Schuler & Cole, 1988). Indeed, deletion of the A+U region from interferon-β gene constructs abolished the enhancing effect of dexamethasone on mRNA turnover (Peppel et al., 1991). All of the above-mentioned genes excluding interferon-β, were reported to require synthesis of new protein(s) to manifest modulation of mRNA turnover by dexamethasone. It therefore seems apparent that in several situations, dexam-
methasone modulates mRNA turnover indirectly through the action of a possibly short-lived protein(s). Since for several of these dexamethasone-controlled mRNAs (uPA, interleukin-1β, MCAF) synthesis of new RNA molecules is also required for its effect, dexamethasone may act by increasing transcription of a gene which encodes the labile protein responsible for enhanced mRNA turnover. The requirement for glucocorticoid receptor, a known transcription modulator (see Jonat et al., 1990), indirectly supports this argument. In this scenario, the induced protein may be an RNase, an A + U RNA-binding protein (reviewed in Hentze, 1991) which signals RNA degradation, or perhaps an undefined accessory factor. The alternate possibility that glucocorticoid receptor itself needs to be continuously synthesised to observe the effect is perhaps less likely, but remains to be experimentally excluded.

Implications of dexamethasone modulation of uPA mRNA stability

The effects of cycloheximide on stability of cytoplasmic uPA mRNA have been investigated in porcine LLC-PK1 cells both in vivo (Altus et al., 1987) and in vitro (Altus & Nagamine, 1991). In log-phase LLC-PK1 cells, following transcriptional induction of uPA mRNA by the peptide hormone calcitonin, the normal half-life of uPA mRNA can be extended from 1 h to > 20 h by cycloheximide treatment (Altus & Nagamine, 1991). Compelling evidence has been presented from the results of in vitro decay experiments to suggest that in LLC-PK1 cells, cycloheximide prolongs uPA mRNA stability by blocking synthesis of a short-lived repressor protein which directly or indirectly associates with uPA mRNA (Altus & Nagamine, 1991). The nature of this protein awaits definition. It is interesting to note that the modulation of uPA mRNA stability in LLC-PK1 cells is the reverse situation to that observed in MAT 13762 cells. In MAT 13762 cells, the steady-state uPA mRNA half-life is > 16 h, which is comparable to that induced in LLC-PK1 cells following cycloheximide treatment. This suggests the intriguing possibility that dexamethasone might induce a similar labile RNA-destabilising protein(s) to that which is constitutively expressed in LLC-PK1 cells.

The above hypothesis does not exclude involvement of other mRNA degradation components. In particular, the mechanism(s) by which dexamethasone enhances mRNA turnover is unlikely to be identical for all mRNAs and in all cell types. This is illustrated by the observation that whilst dexamethasone is known to stabilise c-myc mRNA in leukaemic T-cells (Maroder et al., 1990), c-myc mRNA levels were not altered by this drug in MAT 13762 cells (data not shown). Similarly, in LLC-PK1 cells, c-myc and uPA mRNAs followed distinct degradation pathways (Altus & Nagamine, 1991). The primary sequence element(s) and the short-lived protein(s) responsible for uPA mRNA instability are at present unknown, however the means to identify these determinants may be at hand. The availability of cell model systems in which the proposed unstable repressor protein can be either depleted (by cycloheximide; Altus & Nagamine, 1991) or induced (by dexamethasone; this study), may offer alternate approaches to its eventual identification.
carcinomas is not restricted primarily to the nucleus (Henderson et al., 1992a,b), but that uPA mRNA is also hormonally modulated in the cytoplasm of certain tumour cells. The potential of dexamethasone to repress not only uPA gene transcription (Busso et al., 1987; Medcalf et al., 1988), but also uPA mRNA stability, could provide a more general explanation for the commonly observed repression of uPA mRNA synthesis mediated by this drug in different tumour cell lines.

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