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Temporal Dynamics of Cortisol and Dexamethasone Prevention of Benzo(*a*)pyrene-induced Morphological Transformation of Syrian Hamster Cells

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ABSTRACT

The temporal aspects of the anticarcinogenic action of cortisol were investigated by measurement of the frequency of carcinogen-induced morphological transformation of Syrian hamster embryo cells relative to treatment regimen. Treatment of hamster cells with 10^{-5} to 10^{-11} M cortisol, dexamethasone, corticosterone, 17β-estradiol, progesterone, or testosterone did not result in morphological transformation. However, cortisol or dexamethasone treatment beginning 48 hr before the carcinogen caused a dose-dependent reduction in the transformation frequency associated with benzo(*a*)pyrene [B(a)P] or ultraviolet irradiation treatment. The frequency of B(a)P transformation was reduced 54, 66, and 84% by 10^{-11}, 10^{-10}, and 10^{-9} M cortisol, respectively, without altering colony formation efficiency. Dexamethasone was equipotent as cortisol as an inhibitor of B(a)P, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, or ultraviolet irradiation-induced transformation. Sex steroids, nonsteroid antiinflammatory compounds, and steroids without antiinflammatory activity also reduced the transformation frequency but only at concentrations that reduced colony formation. Cortisol inhibition of transformation increased as steroid exposure occurred closer (48, 24, and 8 hr) yet prior to B(a)P treatment. In contrast, if cortisol was added simultaneously with or up to 72 hr after B(a)P, the frequency of transformation was unaffected. Dexamethasone addition after B(a)P, however, reduced the transformation frequency. Thus, physiological concentrations of cortisol when present prior to carcinogen exposure can prevent carcinogen-induced morphological transformation. The ability of cortisol to inhibit ultraviolet irradiation-induced transformation indicates that the anticarcinogenic activity may be mediated by steroid-induced cellular changes which are independent of metabolic activation of the carcinogen.

INTRODUCTION

Adrenal glucocorticosteroids possess anticarcinogenic activity (2, 10, 11, 17). Prevention of tumor formation by these compounds may result from steroid-induced alterations in aromatic polycyclic hydrocarbon metabolism (9) or from as yet undefined influences upon initiated, promoted, or other stages of carcinogenesis (1, 22-24). One possible mechanism of anticarcinogenesis may involve steroid inhibition of cell proliferation since the prevention of aromatic polycyclic hydrocarbon-induced mouse skin tumors directly correlates with the antiinflammatory potency of the steroid (1). Equimolar concentrations of corticosteroids with divergent antiinflammatory activities, however, cause equivalent inhibition of UV irradiation and B(a)P[2]-induced morphological transformation of Syrian hamster cells (7). Inhibition of transformation, moreover, can occur without inhibition of cell proliferation. This suggests the presence of additional mechanisms besides changes in carcinogen metabolism, antiinflammatory-associated effects, and inhibition of cell proliferation which mediate corticosteroid prevention of carcinogenesis. The present investigation further examines the anticarcinogenic activities of cortisol, the major circulating glucocorticosteroid, through a comparison of the transformation-inhibitory activity of cortisol when added at physiological concentrations to Syrian hamster cells commencing 48 hr before to 72 hr after carcinogen exposure.

MATERIALS AND METHODS

Chemicals. Cortisol, dexamethasone, 11-deoxycortisol, corticosterone, testosterone, progesterone, 17β-estradiol, indomethacin, and salicylic acid were purchased from Sigma Chemical Co. (St. Louis, Mo.). B(a)P was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Cortisol, dexamethasone, 11-deoxycortisol, and corticosterone were initially dissolved in acetone and stored at −20°, and all subsequent dilutions were made in DMEM-10% FBS. Indomethacin and salicylic acid were dissolved in DMEM-10% FBS and stored at −20°. Testosterone, progesterone, and 17β-estradiol were initially dissolved in ethanol, diluted in DMEM-10% FBS, and stored at −20°. For each experiment, fresh stock solutions of B(a)P were prepared by dissolving the carcinogen in acetone and subsequently diluting it in DMEM-10% FBS.

Cells and Cell Culture. Syrian hamster embryo cells were isolated from 13- to 14-day fetuses, frozen in 2.0-ml aliquots of 5×10^6 cells/ml in DMEM-10% FBS: 10% dimethyl sulfoxide, and stored at −170° (4). For each experiment, cells were thawed, seeded in DMEM-10% FBS, and grown in 100-mm plastic Petri dishes in a humidified atmosphere of 90% air: 10% CO_2 at 37° (7). After 3 days, secondary cultures were obtained by seeding 2.5×10^6 cells/100-mm dish in 10 ml DMEM-10% FBS.

Transformation Assay. The quantitative hamster embryo cell transformation assay was performed as described (4, 7). Briefly, 300 secondary hamster cells at 48 hr of growth together with 6×10^4 X-irradiated (2000 R) cells from a sister culture were seeded per 60-mm dish in 4 ml DMEM-10% FBS and treated with UV radiation (5) or chemical carcinogen (6) 24 hr later. Twelve dishes in each experiment were treated with carcinogen and/or other compounds.

Control and carcinogen-treated cells were incubated in a water-saturated atmosphere of 90% air: 10% CO_2 for 7 days, at which time

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[2] The abbreviations used are: B(a)P, benzo(*a*)pyrene; DMEM-10% FBS, Dulbecco's modification of Eagle's minimal essential medium containing 3×10^{-6} M thymidine, 3×10^{-5} M hypoxanthine, and 10% fetal bovine serum (6); MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; I_{50}, concentration reducing the transformation frequency 50%.

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the cells were stained with Giemsa. Morphological transformation was scored as those colonies which exhibited crisscrossed and piled-up cells not seen in control colonies. The frequency of morphological transformation was expressed as the total number of transformed colonies per dish. The frequency of colony formation was determined by dividing the average number of colonies (>2 mm) per dish by the number of cells seeded per dish, multiplied by 100.

Steroids and related antiinflammatory compounds were added, at the indicated final concentrations, 48 hr before to 72 hr after carcinogen treatment. When each of the test compounds was added to the medium at various times prior to the carcinogen, the indicated concentrations were also included in the fresh medium used for the seeding of cells and for the addition of carcinogen 24 hr later. One treatment was administered to the cells in experiments where the compound was added simultaneously with or at various times after carcinogen treatment. Acetone (control) or carcinogen treatment was always applied 24 hr after cell seeding.

RESULTS

Cortisol, sex steroids, and related compounds at concentrations of 10^{-5} to 10^{-8} M showed concentration-dependent toxicity as evidenced by a progressive decline in hamster cell colony formation (Chart 1). No transformation was observed after treatment of hamster cells with these cytotoxic steroid concentrations as well as at lower and noncytotoxic concentrations down to and including 10^{-12} M. In contrast, cell transformation was inhibited by the antiinflammatory steroids, cortisol, dexamethasone, and corticosterone, when the cells were first exposed to the steroids 48 hr prior to carcinogen (Table 1). The addition of 10^{-11} M cortisol or dexamethasone, a potent antiinflammatory synthetic methylfluorinated derivative of cortisol, reduced the number of B(a)P-transformed colonies by 52 and 59%, respectively, without affecting colony formation (Table 1). Corticosterone, a comparatively weak antiinflammatory naturally occurring steroid, did not affect colony formation or cell transformation at 10^{-11} M. However, at 10^{-9} M, corticosterone reduced the number of transformed colonies by 41% independent of any changes in colony formation. 11-Deoxycortisol, a substrate precursor for cortisol without antiinflammatory activity, reduced B(a)P transformation by 56% but also reduced the frequency of colony formation by 24%. Similar results were noted for indomethacin and salicylic acid, 2 nonsteroid compounds with potent antiinflammatory actions. Progesterone, testosterone, and 17β-estradiol also inhibited cell transformation 52 to 74% but at concentrations which also reduced colony formation 40 to 47% (Table 1). At concentrations intermediate to those shown in Table 1, inhibition of transformation was also observed only in conjunction with inhibition of colony formation, *i.e.*, at concentrations enhancing cell killing.

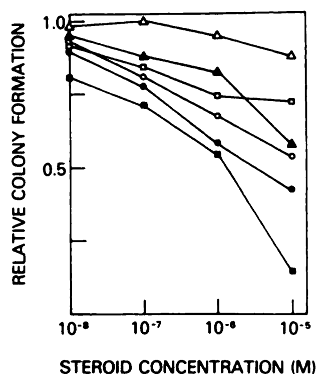

Chart 1. Comparative inhibitory activity of cortisol, sex steroids, and other related compounds on the proliferation of Syrian hamster cells. Secondary hamster cells were seeded on an irradiated feeder layer as described in "Materials and Methods." Cortisol (○), dexamethasone (●), corticosterone (△), 17β-estradiol (▲), progesterone (■), or testosterone (□) were added at the indicated concentrations 24 hr later. After 7 days, cell colonies were counted and scored for morphological transformation. The colony formation frequency of the acetone-treated (control) cells for the 4 experiments ranged from 22.4 to 28.9%. The data shown are in each case the mean colony formation frequency of 4 experiments normalized to the frequency of the nontreated cells. No transformed colonies were observed after the addition of any test compound at the indicated concentration as well as at 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-12} M.

Table 1

B(a)P transformation of Syrian hamster cells exposed to a steroid or nonsteroid commencing 48 hr before carcinogen treatment

Treatment	Test compound (M)	Colony (%) formation[a]	Transformed colonies/dish[a]	% of inhibition of transformation
Acetone control (<0.5%)	0	31.6 ± 0.4	0	
B(a)P (2.5 μg/ml)	0	24.0 ± 0.4	2.7 ± 0.2	0
B(a)P + cortisol	10^{-11}	25.2 ± 0.5	1.3 ± 0.3[b]	52
B(a)P + dexamethasone	10^{-11}	24.8 ± 0.4	1.1 ± 0.2[b]	59
B(a)P + corticosterone	10^{-11}	23.9 ± 0.6	2.9 ± 0.3	0
	10^{-9}	24.2 ± 0.3	1.6 ± 0.3[b]	41
B(a)P + 11-deoxycortisol	10^{-9}	24.6 ± 0.4	3.0 ± 0.4	0
	10^{-7}	18.3 ± 0.3[c]	1.2 ± 0.1[b]	56
B(a)P + indomethacin	10^{-9}	22.6 ± 0.6	2.8 ± 0.4	0
	10^{-7}	14.2 ± 0.2[c]	1.9 ± 0.3[b]	30
B(a)P + salicylic acid	10^{-9}	21.7 ± 0.4	3.2 ± 0.5	0
	10^{-7}	15.8 ± 0.4[c]	2.3 ± 0.2	15
B(a)P + progesterone	10^{-11}	21.4 ± 0.3	3.0 ± 0.1	0
	10^{-9}	14.1 ± 0.2[c]	0.9 ± 0.2[b]	67
B(a)P + testosterone	10^{-11}	23.9 ± 0.4	2.6 ± 0.2	0
	10^{-8}	12.7 ± 0.2[c]	0.7 ± 0.2[b]	74
B(a)P + 17β-estradiol	10^{-11}	25.9 ± 0.3	3.1 ± 0.4	0
	10^{-8}	14.3 ± 0.4[c]	1.3 ± 0.2[b]	52

[a] Mean ± S.E. of 3 to 5 experiments.
[b] $p < 0.05$ [*versus* transformation/dish for B(a)P-treated cells].
[c] $p < 0.05$ [*versus* colony formation frequency for B(a)P-treated cells].

Table 2

UV and MNNG transformation of Syrian hamster cells treated with antiinflammatory steroids commencing 48 hr before carcinogen

Treatment	Test compound (M)	% of colony formation[a]	Transformed colonies/dish[a]	% of inhibition of transformation
Acetone control (0.5%)	0	33.4 ± 0.4	0	
UV irradiation (5 J/sq m)	0	20.1 ± 0.3	1.3 ± 0.2	0
UV + cortisol	10^{-10}	22.4 ± 0.4	0.6 ± 0.1[b]	54
UV + dexamethasone	10^{-10}	19.6 ± 0.2	0.1 ± 0.1[b]	93
MNNG (0.25 μg/ml)	0	18.6 ± 0.2	1.2 ± 0.1	0
MNNG + cortisol	10^{-9}	17.4 ± 0.3	0.1 ± 0.1[c]	92
MNNG + dexamethasone	10^{-9}	16.8 ± 0.4	0.2 ± 0.1[c]	78
MNNG + corticosterone	10^{-9}	17.2 ± 0.5	0.8 ± 0.1[c]	34

[a] Mean ± S.E.
[b] $p < 0.05$ [*versus* transformation/dish of UV-treated cells].
[c] $p < 0.05$ [*versus* transformation/dish of MNNG-treated cells].

When cortisol or dexamethasone, at 10^{-10} M, were added 48 hr prior to UV irradiation of hamster cells, transformation was inhibited 54 and 93%, respectively (Table 2). Similarly, 10^{-9} M cortisol or dexamethasone also inhibited MNNG transformation by 92 and 78%, without altering colony formation. Corticosterone (10^{-9} M), as observed with B(a)P transformation, was a weaker inhibitor than either cortisol or dexamethasone reducing the MNNG transformation frequency by 34%.

Chart 2 demonstrates that treatment with cortisol or dexamethasone at 10^{-11} to 10^{-9} M, beginning 48 hr prior to B(a)P

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addition, inhibited the transformation frequency in a dose-dependent manner. Similar inhibition of UV transformation was observed with both steroids. Equivalent inhibition of transformation occurred with (Chart 2) or without (Table 3) feeder cells, demonstrating the direct effect of cortisol and dexamethasone on the target cells. Cortisol and dexamethasone, moreover, have approximately the same potencies for the inhibition of B(a)P or UV transformation when the I_{50}s are compared. Cortisol I_{50}s were 9.0×10^{-12} M and 2.0×10^{-11} M compared with dexamethasone I_{50}s of 6.8×10^{-12} M and 8.0×10^{-12} M for B(a)P- and UV-induced transformation, respectively. The I_{50} of corticosterone (2.0×10^{-9} M), however, was about 100-fold greater than that of either cortisol or dexamethasone for B(a)P transformation. The I_{50} of 11-deoxycortisol for B(a)P transformation was 6.5×10^{-8} M, a concentration that also reduced colony formation.

The temporal relationships of cortisol and dexamethasone inhibition of B(a)P-induced transformation are summarized in Chart 3. The addition of 10^{-11} M cortisol for 48, 24, or 8 hr prior to B(a)P treatment resulted in increasing reduction in the number of transformed colonies. Similar results were observed with 10^{-11} M dexamethasone. Cortisol (10^{-11} M) when added simultaneously with (0 hr) or 24, 48, or 72 hr after B(a)P did not alter the transformation frequency (Chart 3). Cortisol also did not inhibit cell transformation when added at various times after UV irradiation. On the other hand, the addition of 10^{-11} M dexamethasone simultaneously with or 24 or 48 hr after B(a)P reduced the transformation frequency by 46, 31, and

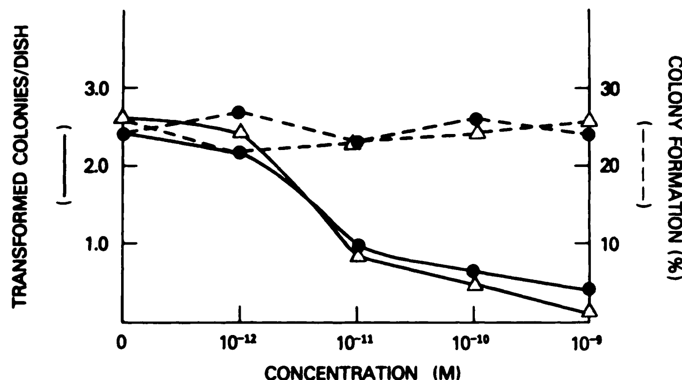

Chart 2. Cortisol (●) and dexamethasone (△) dose-dependent inhibition of B(a)P transformation of Syrian hamster cells. Steroids were added beginning 48 hr before 2.5 μg B(a)P per ml as described in "Materials and Methods." Each *point* is the mean of 2 separate experiments whose standard errors did not exceed 10%.

Table 3

Steroid inhibition of B(a)P transformation of Syrian hamster cells grown in the absence of feeder cells[a]

Treatment[b]		Colony formation (%)[c]	Transformed colonies/ dish[c]	% of inhibition of transformation
Acetone control (0.5%)		18.4 ± 0.4	0	
B(a)P	2.5 μg/ml	13.7 ± 0.3	1.7 ± 0.1	0
B(a)P + cortisol	10^{-10} M	14.2 ± 0.2	0.6 ± 0.1[d]	65
B(a)P + dexamethasone	10^{-10} M	13.2 ± 0.2	0.4 ± 0.1[d]	77

[a] Endothelial cell growth supplement (Collaborative Research Inc., Waltham, Mass.) was included in the medium at 50 μg/ml in place of feeder cells (5).
[b] Steroid treatment commenced 48 hr prior to B(a)P.
[c] Mean ± S.E.
[d] $p < 0.05$ [*versus* transformed colonies/dish for B(a)P-treated cells].

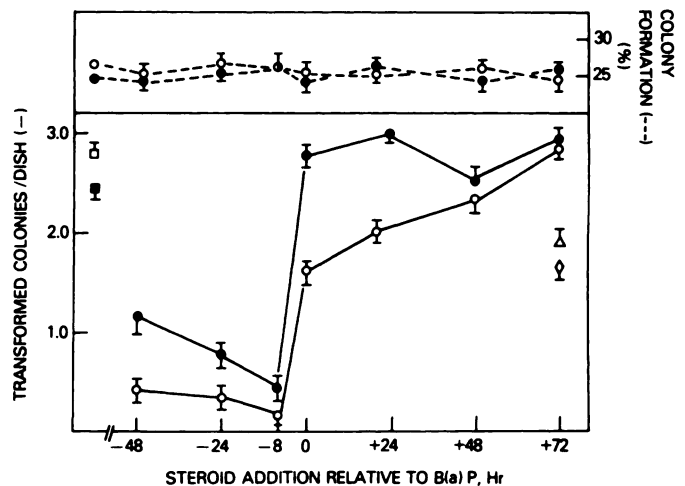

Chart 3. Temporal relationships between cortisol (●) or dexamethasone (○, △, ◇) treatment and the frequency of B(a)P transformation of Syrian hamster cells. Secondary cells were seeded for colony formation 1 day (24 hr) prior to receiving 2.5 μg B(a)P per ml (0 hr), and the transformation frequency, [cells to be treated with cortisol (■); cells to be treated with dexamethasone (□)] was determined 7 days after seeding of the cells. 10^{-11} M steroid treatment (●, ○) commenced 2 days (−48 hr) before B(a)P and was maintained throughout the experiment by inclusion in the medium when cells were seeded for colony formation and in the medium added for B(a)P treatment. 10^{-9} and 10^{-10} M cortisol did not affect the transformation frequency when added after B(a)P and the data points are not shown. △ and ◇, results of treating the hamster cells with 10^{-10} and 10^{-9} M dexamethasone at +72 hr.

17%, respectively (Chart 3). At 72 hr after B(a)P treatment, 10^{-11} M dexamethasone was ineffective as an inhibitor of transformation, but increasing dexamethasone to 10^{-10} or 10^{-9} M reduced the number of transformed colonies per dish by 36 and 43%, respectively (Chart 3). Reduction in the frequency of transformed colonies at noncytotoxic steroid concentrations throughout this investigation occurred without noticeable diminution in colony size or change in morphology other than the prevention of morphological transformation.

DISCUSSION

This study demonstrates that cortisol, the major circulating adrenal glucocorticosteroid [3×10^{-8} M in human plasma (15)], as well as other related glucocorticosteroids at physiological concentrations, can prevent UV irradiation- and chemical carcinogen-induced morphological transformation. The inhibition of transformation independent of a reduction in cell proliferation as measured by cell colony formation suggests a high degree of specificity for the anticarcinogenic activity of these steroids. Rabinowitz *et al.* (18) have reported that cortisol can stimulate the transformation of Syrian hamster cells when added 24 hr after polyoma virus. Our data indicate that the anticarcinogenic actions of cortisol exemplified by inhibition of UV irradiation- and chemical carcinogen-induced morphological transformation requires cortisol addition to the cells prior to carcinogen exposure. Not surprisingly, these findings indicate that the control mechanisms of viral as opposed to UV and chemical carcinogens may be different and/or independent of each other. Cortisol and dexamethasone have also been shown by others to inhibit carcinogen- as well as phorbol diester-promoted morphological transformation of hamster cells (19). In contrast, cortisone and 17β-estradiol induce transformation

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and, along with dexamethasone, synergistically increase X-irradiation transformation of murine C3H 10T½ cells (13, 14). Such divergent observations may reflect methodological, culture, cell cycle, and other molecular differences, emphasizing the need to further characterize the target cells, steroidal concentrations, and temporal interrelationships in various systems of neoplastic transformation.

Previous reports (22-24) have shown that the ability of antiinflammatory steroids to inhibit the phorbol ester-mediated promotional stage of polycyclic hydrocarbon-induced skin carcinogenesis directly correlated with the relative antiinflammatory potencies of the glucocorticosteroids. Dexamethasone is 20 to 30 times more potent as an antiinflammatory compound than is cortisol (15). Both steroids, however, are equipotent as inhibitors of UV- and chemical carcinogen-induced transformation when added prior to the carcinogen. Therefore, other properties of these compounds, in addition to the presence of the steroid nucleus and antiinflammatory activity, are responsible for their actions as anticarcinogens. Moreover, cortisol and dexamethasone both inhibit B(a)P-, MNNG-, and UV-induced transformation which indicates that their anticarcinogenic actions can occur at points or steps in carcinogenesis in addition to carcinogen metabolism.

Dexamethasone, but not cortisol, can inhibit the morphological transformation of hamster cells if added after carcinogen exposure. Other studies have shown that dexamethasone can revert certain neoplastic phenotypic changes, such as the loss of fibronectin and procollagen synthesis by SV40-transformed human fibroblasts to a pattern associated with normal cells (8). The reversion may be nonpermanent requiring the continued presence of the steroid. Therefore, the inhibition of B(a)P transformation by dexamethasone when administered after carcinogen exposure of hamster cells may be a phenotypic reversion of the piled-up, haystack appearance of transformed colonies to the bipolar orientation, a characteristic of nontransformed cells. This latter anticarcinogenic activity of dexamethasone may be related to its antiinflammatory properties, which are more potent than those of cortisol.

Cortisol can induce a variety of cellular alterations including: decreased DNA and hyaluronic acid synthesis (16); increased thymidine incorporation into DNA (26); decreased transfer RNA methylase and protein synthesis (3); decreased plasminogen activator (protease) synthesis (27); increased translation by facilitating mRNA ribosome attachment (21); inhibition of arachidonic acid release from cell lipids resulting in inhibition of prostaglandin release (12); and decreased transport of 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose (25). Some changes such as the decrease in 2-deoxy-D-glucose and uridine uptake and the decrease in thymidine incorporation in chicken embryo fibroblasts occur rapidly (<2 hr) after cortisol treatment (20). These myriad cortisol-mediated effects on cellular processes suggest that the anticarcinogenic action of glucocorticosteroids may be a culmination of a combination of multiple cellular and molecular events. The events and interactions, moreover, may be quite complex *in vivo* as a result of the physiological temporal (diurnal) fluctuations (15) of the adrenocorticosteroids.

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