Potentiation of Glucocorticoid Activity by 5β-dihydrocortisol: Its Role in Glaucoma
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Potentiation of Glucocorticoid Activity by 5β-Dihydrocortisol: Its Role in Glaucoma

Abstract. 5β-Dihydrocortisol potentiated the threshold level (the smallest dose producing a measurable effect) of topically applied cortisol (0.02 percent) and dexamethasone (0.003 percent) in causing nuclear translocation of the cytolsolic glucocorticoid receptor in rabbit iris-ciliary body tissue. 5β-Dihydrocortisol accumulates in cells cultured from trabecular meshwork specimens from patients with primary open-angle glaucoma, but not in similar cells derived from nonglaucomatous patients. In view of the sensitivity of patients with primary open-angle glaucoma to the effects of glucocorticoids in raising intraocular pressure, this potentiation may be responsible for the steroid sensitivity and for the ocular hypertension seen in this disorder.

Primary open-angle glaucoma (POAG) is the most common form of glaucoma and a major cause of blindness. Most patients with this disorder show a marked sensitivity to the effects of topical and systemic glucocorticoids in raising intraocular pressure (1). This suggests that endogenous cortisol plays a role in the ocular hypertension found in POAG.

We reported earlier that cortisol metabolism is altered in cells cultured from specimens obtained by trabeculectomy from patients with POAG (2). These cells accumulated 5β-dihydrocortisol and, to a lesser extent, 5α-dihydrocortisol—intermediates not found in similar cells derived from non-POAG patients. In view of the sensitivity of POAG patients to the effect of glucocorticoids in raising intraocular pressure, we undertook a study of the biologic activity of dihydrocortisol. Nuclear translocation of the cytoplasmic glucocorticoid receptor in rabbit iris-ciliary body tissue was used as a test system. Specific glucocorticoid target cells have been identified in the outflow pathway region of the rabbit by both biochemical and autoradiographic techniques (3). After topical and systemic administration of active glucocorticoids, there was a loss of glucocorticoid receptors from the cytoplasm with a concomitant gain in the nucleus (4). The median effective dose of various steroids for translocation of the glucocorticoid receptor to the nucleus was found to be correlated with the activity of the steroids in raising intraocular pressure (4).

We now report that 5β-dihydrocortisol potentiates the cortisol- and dexamethasone-induced translocation of the cytoplasmic glucocorticoid receptor to the nucleus. This translocation is an early and necessary event in the action of steroid hormones.

Fig. 2. Potentiation of glucocorticoid activity by cortisol metabolites. Glucocorticoid receptor concentration in cytosol was measured after topical (ocular) administration of threshold levels of cortisol (0.02 percent) and dexamethasone-21-phosphate (0.003 percent), with and without steroid metabolites. The values shown represent the average of at least two separate experiments, with six animals used for each (three control and three experimental). Paired groups of animals were used to control for possible variations due to different batches of animals and day-to-day biological fluctuations. One group of three animals each received threshold levels of glucocorticoids alone (solid bars): (A) through (D) cortisolol and (E) dexamethasone-21-phosphate. The other group of three animals each received threshold levels of one of these glucocorticoids with the test metabolites (open bars). The test metabolite was given 2 hours and again 1 hour before the animal was killed. The threshold level of glucocorticoid was administered 1 hour before the animal was killed. The test metabolites and threshold glucocorticoids (in parentheses) were: (A) 1 percent 5β-tetrahydrocortisol (cortisolol); (B) 1 percent 5α-tetrahydrocortisol (cortisolol); (C) 1 percent 5β-dihydrocortisol (cortisol); (D) 1 percent 5β-tetrahydrocortisol (cortisolol); (E) 1 percent 5β-dihydrocortisol (dexamethasone-21-phosphate).

Young New Zealand rabbits weighing less than 2 kg were used as the experimental animals. A control group and an experimental group of three animals each were used in each experiment. The steroids were suspended in phosphate-buffered saline (PBS) by homogenization with a Teflon pestle; this produced a fine suspension of the steroids that minimized corneal irritation. One hour after the last steroid treatment the animals were killed, and the iris-ciliary body was removed, homogenized, and assayed for cytosolic glucocorticoid receptor concentration by determining the specific binding of [3H]dexamethasone (4). The concentration of labeled dexamethasone and the conditions of incubation were sufficient to saturate unbound receptors and to exchange (more than 90 percent) with receptor-bound nonlabeled cortisol or corticosterone (4).

Figure 1 shows the amount of receptor remaining in the cytoplasm after treatment with different steroids (5). Animals

Fig. 1. Glucocorticoid receptor concentration in the cytosol of rabbit iris-ciliary body after topical (ocular) administration of (A) vehicle (PBS), (B) 1 percent 5β-tetrahydrocortisol, (C) 1 percent 5α-tetrahydrocortisol, (D) 1 percent 5β-dihydrocortisol, (E) 1 percent cortisol, and (F) 0.1 percent dexamethasone-21-phosphate. The values shown represent the averages of at least two separate experiments. In each experiment, duplicate measurements were made on pooled tissue from at least three animals. In (A) the standard deviation was determined from seven separate experiments and is indicated on the bar graph. The animals were killed 1 hour after topical application of 25 μl of the indicated steroid solution. When there was no response (A through D), two applications of test solutions were used, at 2 hours and at 1 hour before the animal was killed.
Normal Cells of Patients with High Cancer Risk Syndromes

Lack Transforming Activity in the NIH/3T3 Transfection Assay

Abstract. Oncogenes capable of transforming NIH/3T3 cells are often present in human tumors and tumor cell lines. Such oncogenes were not detected in normal fibroblast lines derived from patients with several clinical syndromes associated with greatly increased cancer risk. Thus, germ-line transmission of these oncogenes does not appear to be the predisposing factor responsible for these high cancer risk syndromes.

The development of DNA-mediated transfection techniques (1) and cell lines capable of detecting transforming DNA sequences (2) has led to the detection of oncogenes in many human tumors and tumor cell lines (3, 4). These genes have arisen from well-conserved normal cellular genes or proto-oncogenes. Genetic changes as small as point mutations (5), as well as DNA rearrangements such as transpositions (6), gene amplification (7), and chromosomal translocations (8), have all been implicated in oncogene activation. Three related oncogenes have been detected at high frequency in the NIH/3T3 transfection assay (4, 9, 10). Each of these genes is related to the ras family of onc genes (11), cellular sequences that have been transduced by retroviruses to form Harvey, BALB, and Kirsten murine sarcoma viruses. The onc genes of these viruses are essential for their tumor-inducing potential.

While most forms of cancer occur sporadically, clinical observations have revealed the existence of certain familial aggregations of cancer involving specific target organs. Other familial syndromes are associated with a strikingly increased risk of cancer characterized by onset at an early age and tumors affecting tissues of more than one developmental type (12). Such clinical syndromes provide the opportunity to test whether the germ-line transmission of oncogenes capable of registering in the NIH/3T3 transfection assay might account for these patients' genetically determined high risk of cancer.

High molecular weight DNA was prepared from fibroblast cultures derived from skin of patients with various clinical conditions associated with a high risk of cancer (Table 1). The largest group comprised affected individuals from five families with Gardner's syndrome. This syndrome is associated with a dominant trait for susceptibility to colon cancer as well as a striking familial predisposition to benign neoplasms of bone, soft tissue, and skin (13). Essentially 100 percent of such affected individuals develop colon cancer in the absence of clinical intervention (13). In addition, we studied fibroblasts from tumor-bearing members of families with site-specific tumor aggregations with apparent autosomal transmission (12, 14). Patients affected with other syndromes associated with strikingly increased cancer risk, including xeroderma pigmentosum and von Recklinghausen's syndrome, were studied as well (12).

DNA transfection was performed as described earlier (10, 15). We observed transforming activity with 20 to 33 percent of colon or lung carcinoma DNA samples analyzed (Table 1). These results compare favorably with the reported prevalence of transforming genes in cell DNA's from human tumors as detected with the NIH/3T3 assay (5, 4, 10). The transforming activity associated with positive tumor cells ranged from 0.2 to 1.3 focus-forming units per plate. Representative transfectants were also tested and shown to contain human repetitive sequences (16), confirming that each had taken up exogenous human DNA. In contrast, we detected no evidence of transforming DNA sequences in any of the skin fibroblast lines from patients with the high cancer susceptibility syndromes analyzed. To ensure that each of the fibroblasts was adequately tested, more than 1000 μg of high molecular weight DNA was tested in each case, with analysis conducted in at least two separate experiments.

Antisera that recognize the 21,000-dalton (p21) gene products of both mouse and human homologs of the ras genes are available (17). Moreover, in human tumor cells that contain activated ras oncogenes, the mobility of the p21 protein can be altered (5). Therefore, we also considered whether any abnormality could be detected in the physical characteristics or level of expression of these proteins in any of the human fibroblast lines analyzed. By use of monoclonal antibodies that detect human p21, we were able to demonstrate a new p21 species with altered electrophoretic mobility in the T24 bladder carcinoma cell line (5). We also examined seven additional human tumor cell lines known to contain activated ras oncogenes. Three of these showed either an additional p21 species or an increase in the relative...