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HORMONAL REGULATION OF BENZO[A]PYRENE METABOLISM IN HUMAN ADRENOCORTICAL CELL CULTURES

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Summary: In cultured fetal human adrenocortical cells, metabolism of the carcinogen benzo[a]pyrene was found to be unresponsive to the xenobiotic inducers 3-methylcholanthrene, benz[a]anthracene and 2,3,7,8-tetrachlorodibenzo-p-dioxin. However, exposure of cultures to the hormone adrenocorticotropin (ACTH) for 48 hours stimulated benzo[a]pyrene metabolism 3-fold. The major metabolite was the 7,8-diol. Other compounds which stimulate the production of adrenocortical cell cyclic AMP (forskolin and cholera toxin) as well as monobutyryl cyclic AMP also increased benzo[a]pyrene metabolism. Human adrenocortical cells thus provide an unusual example of hormonal regulation of the metabolism of a carcinogen.

Generally, the metabolism of polycyclic aromatic hydrocarbons in the liver and in extrahepatic tissues is increased after exposure to various xenobiotic inducers (1). Regulation of polycyclic aromatic hydrocarbon metabolism by hormones has been reported only rarely; in particular, the metabolism of benzo[a]pyrene by the rat adrenal cortex was found to be under the long term regulation of adrenocorticotropin (ACTH) and was unresponsive to the administration of methylcholanthrene (2-4). The partially purified cytochrome P-450 responsible for this activity differed in its properties from the steroidogenic cytochrome P-450 species (2,5). We report here that benzo[a]pyrene metabolism in cultured fetal human adrenocortical cells is also regulated by ACTH and other compounds which stimulate the synthesis of cyclic AMP and does not respond to xenobiotic inducers.

Materials and Methods

Preparation of human fetal adrenocortical cultures: Human adrenocortical cell cultures were prepared by collagenase/DNase digestion from the definitive (adult) zone of the fetal human adrenal cortex, as described in detail previously (6-8). For each experiment, we used pooled cells from the adrenal glands from 4-

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5 abortuses of 16 to 20 weeks gestation, obtained through the cooperation of the Department of Reproductive Medicine, University of California Medical Center, San Diego. No differences in results between different lots of cells were noted.

Primary cultures were prepared by plating cells in a 1:1 mixture F-12 medium and Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Culture dishes were coated with 10 μg/ml fibronectin (9) before plating. After 24 hr, the medium was replaced with medium supplemented with 10% horse serum (Irvine Scientific) and 100 ng/ml bovine brain fibroblast growth factor (FGF) (7).

Purity of the cell population: The primary culture consists largely (>95%) of adrenocortical cells and under appropriate conditions this purity is maintained (7). This was assessed by addition of ACTH, which causes a characteristic morphological response ('retraction' or 'rounding'), involving breakdown of actin stress-fibers (10). Fibroblasts and other non-adrenocortical cells do not show this response.

Defined medium: Adrenocortical cells were grown in serum-containing medium and changed to defined medium prior to use in experiments. The serum-free medium is that formulated for adrenocortical cells by Simonian (11), consisting of DME/F-12 1:1, fatty acid free BSA, transferrin, insulin and FGF.

Addition of non-water-soluble compounds: Non-water-soluble compounds, e.g. benzo[a]pyrene and 3-methylcholanthrene, were added from 1000-fold concentrates in acetone. This concentration of acetone has not been observed to have any effects in a variety of different experiments.

Incubation with [^{3}H]benzo[a]pyrene: Before incubation with benzo[a]pyrene, cells were incubated for 1 hr in defined medium without additions. [^{3}H]benzo[a]pyrene was obtained from New England Nuclear and was stored in hexane under N_2 in the absence of light until required. Periodically the purity of the stock solution was examined by HPLC. Just before use, the hexane solution was evaporated under N_2 and a solution of benzo[a]pyrene in acetone was added to a concentration of 4 mM. This was then added to the incubation medium (see above) to a final benzo[a]pyrene concentration of 4 μM. The incubation was normally for 24 h. Shorter incubation times yielded a lower production of metabolites, but with the same pattern of products. At the end of this period the medium was taken for analysis and the culture was trypsinized for estimation of cell number. Samples of benzo[a]pyrene-containing medium without cells were also incubated as controls. No breakdown of [^{3}H]benzo[a]pyrene was observed in these control incubations.

Assay of benzo[a]pyrene metabolites: The complete medium was assayed without solvent extraction, to avoid loss of polar compounds. The medium was deproteinized prior to analysis by addition of acetonitrile to 80% v/v, filtration through a 0.45 μm Zetapor filter (AMF Co., Seguin, Tx.), dilution with water to 20% acetonitrile v/v, and concentration by loading on an octadecylsilyl loop column. The loop column was then switched in line with a separation octadecylsilyl column and gradient elution from 20 to 100% acetonitrile was performed. Radioactive products were collected using a programmable fraction collector with 0.1 min sample collection during peaks and 1 min sample collection between peaks. Products were evaluated as pmol produced per 10^4 cells per 24 h. Benzo[a]pyrene metabolite standards were obtained from the Chemical Repository of the National Cancer Institute.

Results

[^{3}H]Benzo[a]pyrene was converted by cultured human adrenocortical cells largely to the 7,8-diol with smaller amounts of products in the regions of the quinones, phenols, and polar metabolites (Fig. 1a). This pattern of metabolism

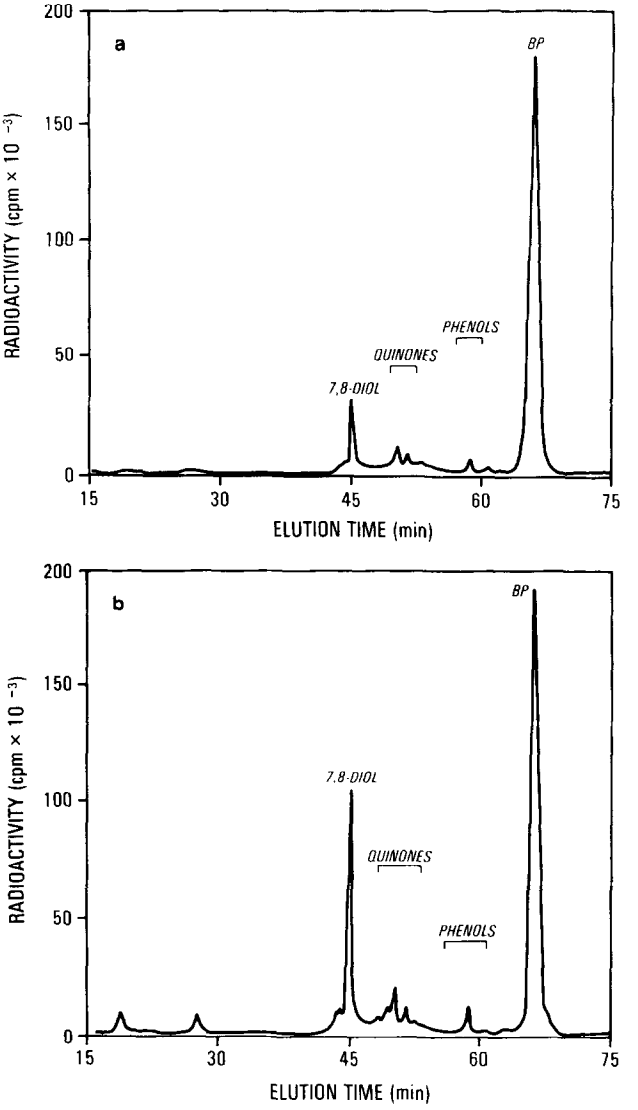

Fig. 1: High performance liquid chromatographic separation of products of metabolism of benzo[a]pyrene in cultured human adrenocortical cells. Cultures were incubated in defined medium containing 4 μM [^{3}H]benzo[a]pyrene as described in Materials and Methods. After 24 h, the medium was taken for analysis as described in the text. (a) (top) Control culture, incubated in defined medium for 48 h prior to incubation with benzo[a]pyrene; (b) (bottom) Culture incubated in defined medium with 1 μM ACTH for 48 h prior to incubation.

has been observed in some established cell lines (12). When human adrenocortical cells were exposed to 1 μM ACTH for 48 h prior to incubation with benzo[a]pyrene, the amount of the 7,8-diol produced increased markedly (Fig. 1b). There were also increases noted in the quinones, phenols, and polar metabolites, but the 7,8-diol remained by far the predominant product.

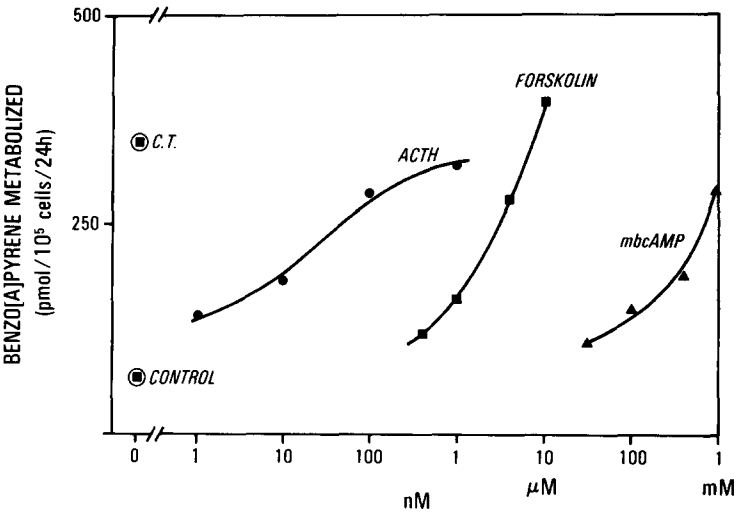

Fig. 2: Stimulation of benzo[a]pyrene metabolism by compounds which raise intracellular cyclic AMP. The indicated concentrations of the compounds were added to cultures in defined medium 48 h prior to the incubation with [^{3}H]benzo[a]pyrene. C.T. = 1 nM cholera toxin; mbcAMP = monobutyryl cyclic AMP. Conversion to the 7,8-diol was then assayed by HPLC as described in the text.

Other compounds which stimulate the production of cyclic AMP also stimulated benzo[a]pyrene metabolism (Fig. 2). Forskolin, cholera toxin, and monobutyryl cyclic AMP, at concentrations that were not cytotoxic, all produced increases in benzo[a]pyrene metabolism with patterns similar to that observed for ACTH, with the 7,8-diol being the major metabolite. The concentrations required were similar to those previously found to stimulate steroidogenesis (10,13).

The time-course of ACTH stimulation of benzo[a]pyrene metabolism was investigated. As previously found for the induction of the steroidogenic enzymes in cultured human adrenocortical cells (6,13), maximal activity was achieved after about 48 h of incubation with ACTH (Fig. 3).

Discussion

In several species, the adrenal cortex has been found to have a fairly high rate of benzo[a]pyrene metabolism (2-4,15-22). Benzo[a]pyrene is metabolized in the adrenal cortex by cytochrome P-450s which are thought to differ from the steroidogenic enzymes. A form of cytochrome P-450 metabolizing polycyclic aromatic hydrocarbons, distinct from the steroidogenic cytochrome P-450s, has

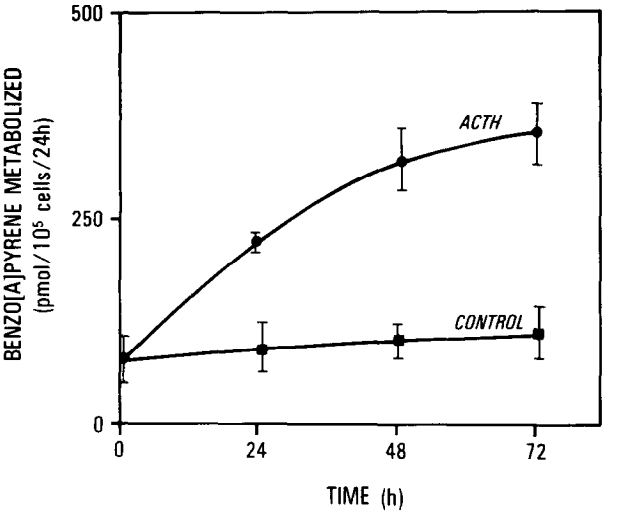

Fig. 3: Time-course of stimulation of benzo[a]pyrene metabolism by ACTH in cultured human adrenocortical cells. Cultures were incubated with 1 μM ACTH in defined medium for the indicated periods prior to the incubation with [^{3}H]benzo[a]pyrene. Conversion to the 7,8-diol was then assayed by HPLC as described in the text.

been partially purified and characterized from the rat adrenal cortex (2,5). Studies indicate no competition for metabolism or enzyme binding between steroids and xenobiotics (23-26). The activities are differentially sensitive to various inhibitors (27,28) and are differentially susceptible to loss after incubation with canrenone (29).

In the present experiments, benzo[a]pyrene metabolism was found to be hormonally regulated in human adrenocortical cells and was unresponsive to xenobiotics. We used cultured fetal human adrenocortical cells, which are a useful system for investigating the control of cytochrome P-450-dependent enzymes (6,13,30). Previously, the regulation of human adrenal xenobiotic metabolism has not been investigated, although two preliminary reports show cytochrome P-450-dependent metabolism of xenobiotics in cultured human adrenocortical cells (31,32). In the rat, both steroidogenesis and benzo[a]pyrene metabolism are coordinately regulated by ACTH (2), and benzo[a]pyrene metabolism is unresponsive to xenobiotic inducers (3,4); but in the guinea pig, mouse, and bovine adrenals, steroidogenic and benzo[a]pyrene-metabolizing activities are differentially regulated by ACTH and xenobiotic inducers (19,26,33-35). Benzo[a]pyrene metabolism in some established cell

lines in culture is stimulated by cyclic AMP (36), and ACTH is known to induce the steroid hydroxylase cytochrome P-450s by stimulating the production of cyclic AMP (37). The ability to experimentally regulate benzo[a]pyrene metabolism by hormones in cell culture provides a useful means of investigating the induction and expression of the cytochrome P-450s involved.

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