Aryl Hydrocarbon (Benzopyrene) Hydroxylase Is Stimulated in Human Lymphocytes by Mitogens and Benz[a]anthracene

Abstract. *A mixed-function oxidase that requires reduced nicotinamide adenine dinucleotide phosphate, is carbon monoxide sensitive, and is drug-metabolizing is present in human lymphocytes and is increased to different levels by treatment with phytohemagglutinin, pokeweed mitogen, and a polycyclic hydrocarbon.*

Microsomal mixed-function oxidases constitute the key enzyme systems for the metabolism of a wide variety of xenobiotics including drugs, pesticides, and chemical carcinogens, as well as certain endogenous compounds, such as steroids (*1*). Aryl hydrocarbon hydroxylase (AHH) is a part of the cytochrome P-450–containing microsomal enzyme system present and inducible in many tissues of different species (*2*), in various cells grown in culture (*3*), and in human placenta (*4*). The hydroxylase is involved in both the detoxification of polycyclic hydrocarbons and in the activation of some to reactive carcinogenic forms (*1*, *3*).

The role of AHH in the detoxification or activation of chemical carcinogens in man is not understood. Furthermore, investigation of microsomal enzymes in humans is hampered by the difficulty of obtaining suitable tissues. We now report evidence that AHH is present and inducible in human peripheral lymphocytes. This finding may further studies on the role of microsomal drug-metabolizing enzymes in chemical carcinogenesis and in drug metabolism in humans, as well as studies on the genetic and environmental factors regulating this enzyme system in the human population.

Although lymphocytes are a relatively easily obtainable tissue, they have little cytoplasm and endoplasmic reticulum. However, after exposure to mitogens, a complex sequence of metabolic and morphologic changes occurs, including cytoplasmic enlargement and proliferation of endoplasmic reticulum (*5*). We therefore utilized both resting and mitogen-treated cells to examine the level and inducibility of AHH. Cells treated with phytohemagglutinin or pokeweed mitogen (PWM) exhibited an increase in AHH activity and a greater sensitivity to AHH induction by benz[*a*]-anthracene (BA).

Lymphocytes were purified from freshly drawn heparinized venous blood by passage through a nylon column and a Ficoll-Hypaque solution (*6*). The resulting cell population was morphologically at least 99 percent lymphocytes; the yield from 500 ml of blood was 400×10^6 to 1000×10^6 small lymphocytes, which yielded approximately 4 to 10 mg of protein. Cells were suspended at a density of 2×10^6 cell/ml in Eagle's minimal essential medium (Gibco) supplemented with 10 percent fetal calf serum, penicillin (100 unit/ml), and streptomycin (100 μg/ml) and were incubated in a 5 percent CO_2, humidified atmosphere. For studies involving resting cells (no mitogen), cells were suspended in medium containing 10 percent autologous plasma instead of fetal calf serum. The medium was supplemented by addition of an equal volume of fresh medium after 48 hours of incubation.

Phytohemagglutinin (Burroughs-Wellcome) was used at a concentration of 2.5 μg per milliliter of cell suspension. Pokeweed mitogen (Gibco) was used at a concentration of 0.01 ml per milliliter of cell suspension. Cells were exposed to mitogens for 48 to 72 hours before exposure to BA. The BA (5 mg) was dissolved in 0.50 ml of acetone and dispersed in 50 ml of fetal calf serum; the resulting suspension was added to 450 ml of Eagle's minimal essential medium. The concentration of BA in the medium was measured spectrophotofluorometrically, with excitation at 340 nm and fluoresence at 398 nm.

For treatment with BA, the cell suspensions were centrifuged at 600*g* for 5 minutes, and the pellet was resuspended in control medium or in medium containing BA. After exposure to BA, the cells were centrifuged at 600*g* for 5 minutes, and the pellet was washed once with Dulbecco's phosphate-buffered saline (Gibco), centrifuged, and homogenized in 0.25*M* sucrose–0.05*M* tris buffer, *p*H 7.5, in a Potter-Elvehjem glass homogenizer. Portions of homogenate were assayed for AHH activity (*3*). Each incubation mixture contained, in a final volume of 1.0 ml, 50 μmole of tris-chloride buffer, *p*H 7.5, 3 μmole of $MgCl_2$, 0.5 mg of reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 100 nmole of benzo[*a*]pyrene. Incubation was usually for 30 minutes at 37°C and was carried out in subdued light. The reaction was stopped with an acetone-hexane mixture, and the fluorescence of the alkali-extractable phase was measured in an Aminco-Bowman spectrophotofluorometer and compared to that of a standard 3-hydroxybenzo[*a*]pyrene solution. Protein concentration was determined by the method of Lowry *et al.* (*7*). The characteristics of the enzyme from phytohemagglutinin-stimulated cells were examined; the production of hydroxylated benzo[*a*]pyrene was linear with respect to time (10 to 30 minutes)

Table 1. Requirements for AHH from phytohemagglutinin-stimulated, BA-treated human lymphocytes. Cells were stimulated with phytohemagglutinin for 48 hours and then exposed to BA for 18 hours. The concentration of BA in the medium was 25 to 30 μM. Cells were collected, pooled, homogenized, and treated as described. One unit of AHH activity catalyzes in 30 minutes the formation of phenolic products with the fluorescence equivalent to that of 1 pmole of 3-hydroxybenzo[*a*]pyrene. Each assay contained 1.6 mg of cellular protein.

Incubation system	Specific activity*
Complete	8.0
No NADPH	1.2
90 percent CO and 10 percent O_2	5.4
7,8-BF ($10^{-4}M$)†	4.0
Prior incubation,‡ then complete	8.8
Prior incubation with trypsin (50 μg), then complete	0.9

* Units per milligram of protein. † 7,8-Benzoflavone (BF) added in 10 μl of dimethyl sulfoxide. ‡ Prior incubation was for 10 minutes at 37°C in a volume of 0.91 ml, containing 50 μmole of tris-chloride buffer, *p*H 7.5, and 3 μmole of $MgCl_2$.

Table 2. Basal and BA-induced AHH activity in resting and mitogen-stimulated human lymphocytes. Cells were treated with phytohemagglutinin (PHA) or pokeweed mitogen (PWM) for 48 to 72 hours, followed by exposure to the inducer (BA) for 16 to 18 hours. Concentration of BA in the medium was 25 to 30 μM. Cells were collected and assayed for AHH activity as described. Values in parentheses indicate the number of different samples on which each assay was performed.

Mitogen	Specific activity*
None (2)	
Control	0.8 ± 0.20
BA	1.5 ± 0.27
PHA (4)	
Control	1.8 ± 0.40
BA	5.4 ± 0.50
PWM (2)	
Control	1.8 ± 0.27
BA	11.5 ± 1.7

* Units per milligram of protein ± standard error of the mean.

and with respect to cellular protein (0.7 to 12 mg) within the ranges used in these experiments.

The requirements for AHH from phytohemagglutinin-stimulated lymphocytes (Table 1) are similar to the requirements of AHH from rat liver microsomes as well as from hamster embryo cells (*1*, *3*). The enzyme has characteristics typical of the microsomal mixed-function oxidases: a requirement for NADPH, inhibition by carbon monoxide, suggesting an involvement of the P-450 hemoprotein, and inhibition by 7,8-benzoflavone, a potent inhibitor of the AHH system (*8*). The protein nature of the catalysis is further indicated by its sensitivity to trypsin digestion.

Activity of AHH in resting, in mitogen-stimulated, and in BA-treated lymphocytes is shown in Table 2. The AHH activity of lymphocytes compared to rat liver microsomes or hamster embryo cells is relatively low. Mitogen treatment alone increases AHH activity about twofold. The difference in AHH between resting and mitogen-treated cells, however, is amplified by treatment with BA. Thus, mitogen-treated cells exposed to BA exhibit a three- to eightfold greater AHH activity than resting cells treated with BA.

The finding of highest AHH activity in PWM-stimulated, BA-treated cells is biochemical evidence consistent with the morphologic observation that PWM stimulates proliferation of endoplasmic reticulum more intensely than does phytohemagglutinin (*9*). Therefore, AHH may be useful for biochemical detection of quantitative changes in the endoplasmic reticulum of lymphocytes at various stages of their differentiation. Purification of lymphocytes by column absorption techniques, as in this study, may preferentially remove "B"-type lymphocytes (*10*). The latter are thought to be the progenitors of antibody-producing plasma cells (*11*) and may be the precursors of the plasmacytoid cells seen in PWM-stimulated cultures (*9*). Our finding of a significantly greater increase in AHH activity after PWM than after phytohemagglutinin stimulation suggests either that B cells are not completely removed by the lymphocyte purification technique, or that blast cells with increased endoplasmic reticulum may arise from PWM stimulation of the remaining T cells.

Investigation of AHH in humans is relevant to several problems. AHH may play a role in chemical carcinogenesis; furthermore, specific inhibitors, such as 7,8-benzoflavone (*8*), or inducers, such as polycyclic hydrocarbons or phenobarbital (*12*), may be useful in reducing the toxic or carcinogenic effects of exposure to certain environmental compounds.

Individual variations in response to drug therapy, susceptibility to drug toxicity, and drug synergism or antagonism may be related to differences in drug-metabolizing enzymes (*13*).

This is the first report of the presence of a drug-metabolizing enzyme system in an easily available human tissue. The detection of other microsomal enzymes in human lymphocytes may require assays more sensitive than those presently available. The study of AHH and other drug-metabolizing enzyme systems in lymphocytes may help to reveal the genetic and environmental factors and the molecular mechanisms involved in the regulation of this enzyme system, which is important in the organism's response to exogenous chemicals.

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Nicotine Blocks the Suckling-Induced Rise in Circulating Prolactin in Lactating Rats

Abstract. *In lactating rats the rapid suckling-induced release of pituitary prolactin into circulating blood is inhibited by a subcutaneous injection of nicotine. This treatment does not block the lesser "stress-induced" rise in prolactin in response to either. Although nicotine may impair milk production by its effect on prolactin release, it does not appear to block milk ejection from the lactating mammary gland.*

Nicotine has been implicated as the constituent of tobacco smoke that is responsible for a high incidence of reproductive disorders during pregnancy and an increased mortality rate in infants (*1*). Until now, no one has specifically investigated the effects of nicotine on the neural events that initiate the suckling-induced rise in circulating prolactin and oxytocin, two hormones which are necessary to maintain milk production and discharge, respectively. It is well established that suckling causes a rapid release of prolactin and oxytocin from the pituitary gland in the rat (*2*). More recently, stress has been shown to cause prolactin output (*3*), and the mechanisms involved in prolactin release after suckling and after exposure to ether have been shown to be different (*4*). We now report that nicotine inhibits the suckling-