Apoptotic mitochondrial dysfunction induced by benzo(a)pyrene in liver epithelial cells: role of p53 and pHi changes.

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Role of p53 and pH_i Changes

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ABSTRACT: How pH_i changes, more specifically alkalinization, affect the apoptotic cascade has yet to be determined. The aim of the present work was to test the involvement of mitochondria in the apoptotic cascade triggered by benzo(*a*)pyrene [B(a)P] and to determine the role of pH_i changes and p53 relative to mitochondria. Our results indicate that B(a)P-induced apoptosis might rely upon a p53-dependent and a pH-sensitive mitochondrial dysfunction.

KEYWORDS: benzo(*a*)pyrene; rat liver epithelial cell; apoptosis; mitochondria; intracellular pH; p53; reactive oxygen species

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), such as benzo(*a*)pyrene [B(a)P], are responsible for important carcinogenic and apoptotic effects, whose mechanisms are still poorly understood. With respect to PAH-induced apoptosis, this phenomenon has been shown to require caspase[1] as well as p53 activation.[2] An important role for reactive oxygen species (ROS) production has been demonstrated in cell death induced by various chemicals.[3] Regarding this latter point, although PAHs, via metabolism through cytochrome 1A1, are well known to induce ROS production, a role for such species in the apoptotic cascade triggered by these compounds remains to be established. Besides ROS, cytoplasmic acidification has also been proposed as an important factor in apoptosis.[4] In this context, it is worth noting that we have shown previously that biphasic perturbations of H^+ homeostasis (i.e., early alkalinization followed by late acidification) were elicited during B(a)P-induced cell death in the rat liver F258 epithelial cell line and that inhibition of those changes afforded a significant cell protection against B(a)P-induced toxicity.[5] However, how pH_i changes, more specifically alkalinization, affect the apoptotic cascade has yet to be determined. The aim of the present work was to test the involvement of mitochondria in

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TABLE 1. Apoptotic effects of benzo(*a*)pyrene (72h) in F258 rat liver epithelial cell line

Cell Treatment	DNA Fragmentation Detected by Hoechst 33342 Staining of Cells (% control population)	Caspase-3–like Activity (% control population)
B(a)P (50 nM)	274.0 ± 1.7%	692.2 ± 69.9%
B(a)P + α-Naphthoflavone (10 μM)	118.3 ± 3.4%[a]	85.6 ± 0.1%[a]
B(a)P + Cariporide (30 μM)	181.2 ± 2.7%[a]	276.6 ± 21.7%[a]
B(a)P + Bongkrekic acid (10 μM)	128.9 ± 2.8%[a]	378.2 ± 26.2%[a]
B(a)P + Pifithrin α (10 μM)	121.1 ± 2.1%[a]	79.6 ± 0.1%[a]

Results were quoted as mean±SEM of three independent experiments. They were expressed as percentage of effects compared to values obtained from control, untreated cells. Inhibitor, B(a)P-treated cells versus control, B(a)P-treated cells, [a]$P<.05$ (ANOVA followed by Student-Newman-Keuls test).

the apoptotic cascade triggered by B(a)P and to determine the role of pH_i changes and p53 relative to mitochondria.

MATERIAL AND METHODS

The F258 rat liver epithelial cell line, cultured in Williams' medium E supplemented with 10% fetal calf serum at 37°C under a 5% CO_2 atmosphere, were treated during the exponential phase with 50 nM B(a)P and/or the different inhibitors tested (applied to cells 2 h prior to PAH exposure) for various treatment times.

Microscopical detection of apoptosis was performed in both floating and adherent cells, using Hoechst 33342 (0.5 μg/mL) labeling. Caspase activity was measured in cell lysates using the fluorogenic substrate DEVD-AMC.

Mitochondrial potential and ROS production were measured by flow cytometry using $DiOC_6(3)$ (50 nM) and dihydroethidium (DHE; 5 μM), respectively.

RESULTS AND DISCUSSION

Results quoted in TABLE 1 show that treatment of F258 cells by B(a)P (50 nM, 72 h) led to a significant apoptotic effect (as detected by Hoechst 33342 staining and caspase-3–like activity) compared to untreated counterparts. Using α-naphthoflavone (10 μM; a CYP1A1 inhibitor) and cariporide (30 μM; an inhibitor of Na^+/H^+ exchange which has been shown to inhibit PAH-induced pH_i change[5]), we demonstrated the involvement of B(a)P metabolism and associated pH_i changes in the apoptotic cascade elicited by B(a)P. Furthermore, a role for mitochondria and the tumor suppressor p53 was suggested since B(a)P-induced apoptosis was markedly inhibited by bongkrekic acid (10 μM) and pifithrin α (10 μM), respectively. In order to gain more insight into a putative effect of B(a)P on mitochondrial function, we decided to measure mitochondrial membrane potential ($\Delta\Psi m$) using $DiOC_6(3)$ staining of cells. As illustrated in FIGURE 1, a mitochondrial hyperpolarization was

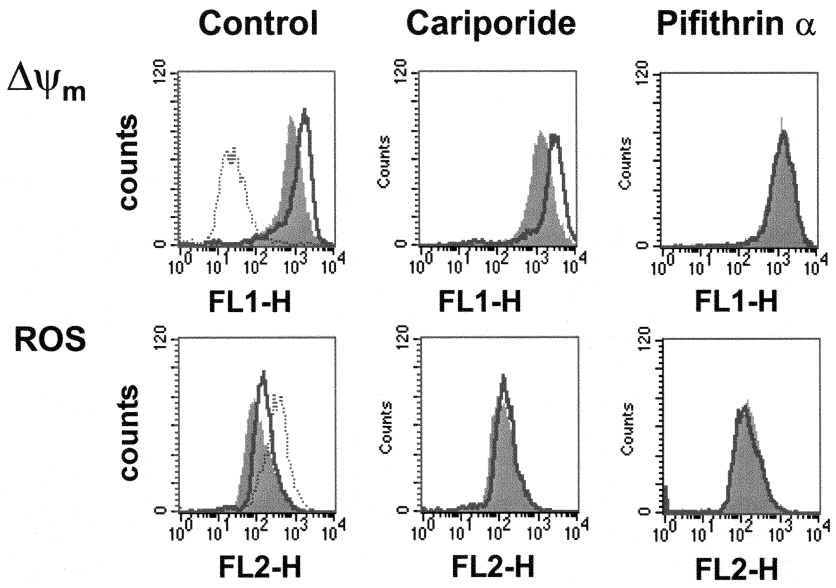

FIGURE 1. Effects of B(a)P (50 nM, 48 h) on mitochondrial membrane potential and ROS production in F258 cells. Mitochondrial membrane potential (ΔΨm) and ROS production were measured using $DiOC_6(3)$ and DHE staining of the cells, respectively. Illustrated histograms were representative of three independent experiments. (*Filled, grey peak*) Control, untreated cells; (*open, black peak*) B(a)P-treated cells; (*open, dotted peak*) positive control cells (i.e., in the presence of FCCP [50 μM] and menadione [100 μM] when $DiOC_6(3)$ and DHE were used, respectively).

detected following a 48-h treatment, in contrast to the well described depolarization observed upon FCCP application. We next tested the effects of B(a)P upon ROS production. FIGURE 1 shows that B(a)P induced a marked production of ROS in F258 cells, as pointed out by a rightward shift of the fluorescent peak, similar to that observed with menadione, a known pro-oxidant molecule. This production was found to be inhibited by bongkrekic acid (not shown). In order to test the involvement of p53 and of the previously observed B(a)P-induced alkalinization in the changes described above, we evaluated the effects of pifithrin α (10 μM) and cariporide (30 μM). Our data show that whereas pifithrin α prevented both hyperpolarization and ROS production, cariporide was effective only upon ROS production (FIG. 1). Altogether, our results indicate that B(a)P-induced apoptosis might rely upon a p53-dependent and a pH-sensitive mitochondrial dysfunction.

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