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Polycyclic Aromatic Hydrocarbons Inhibit the Activity of Acetylcholinesterase Purified from Electric Eel

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Polycyclic aromatic hydrocarbons (PAHs) are formed during the incomplete combustion of fossil fuels, wood and municipal waste incineration, from internal combustion engines, and from various food cooking operations and are common environmental contaminants which have been detected in surface waters, sediments, soils, plants, and both rural and urban air. In this study, we have shown that, for the first time, *in vitro* addition of PAHs dose-dependently inhibited the activity of acetylcholinesterase purified from electric eel in a competitive manner. The PAHs containing 3 or higher aromatic rings showed the highest inhibitory effect with the IC_{50} values between 2 and 6 ppm. Among the PAHs tested, chrysene and pyrene exhibit the highest and lowest potency with IC_{50} values of 2.40±0.04 and 5.22±0.38 ppm, respectively. PAHs with lower number of aromatic rings, such as naphthalene, acenaphthylene and fluorene, and oxygenated PAHs, such as anthraquinone and xanthone, showed no or slight inhibition of the acetylcholinesterase activity.

Key Words: **acetylcholinesterase; polycyclic aromatic hydrocarbons; competitive inhibitor**

The primary function of acetylcholinesterase (AChE; EC 3.1.1.7) is to terminate the action of acetylcholine (ACh) at the junctions of the various cholinergic nerve endings with their effector organs or postsynaptic sites. Chemicals that inhibit AChE are names anticholinesterase agents. They cause the free, unbound ACh to accumulate at cholinergic receptor sites and thus resulted in the overstimulation of muscarinic and nicotinic cholinergic receptors in the central and peripheral nervous system. Environmental pollutants, including organophosphate and carbamate (1); heavy metals such as copper, mercury, lead, iron, nickel (2) and chemicals of other structural classes (3) have been shown to inhibit AChE activity.

Polycyclic aromatic hydrocarbons (PAHs) are common environmental contaminants which have been detected in surface waters, sediments, soils, plants, and both rural and urban air. PAH compounds are formed during the incomplete combustion of fossil fuels, wood and municipal waste incineration, from internal combustion engines, and from various food cooking operations but may also be formed to a small extent by microbes and plants (4,5). Most, if not all, PAHs induce biological responses in invertebrate, fishes and mammals (6-8). Although numerous studies have examined the deleterious health effects of chemicals within this class, their effect on AChE has not yet been studied.

In this study, we have presented evidences showing that, for the first time, several PAHs dose-dependently inhibited the activity of AChE in a competitive manner.

METHOD

1. All polycyclic aromatic hydrocarbons were purchased from Sigma Chem. Co. (St. Louis, MO, USA) except that fluoranthene was purchased from Aldrich Chem. Co. (Milwaukee, WI, USA) and nitropyrene from Tokyo Kasei Chem. Co. (Tokyo, Japan). All other chemicals and enzyme used in acetylcholinesterase activity measurement were purchased from Sigma Chem. Co. (St. Louis, MO, USA).

2. Acetylcholinesterase activity measurement - The enzymatic activity of acetylcholinesterase (AChE; EC 3.1.1.7) purified from electric eel (type V-S) was determined by the method of Ellman *et al* (9) using acetylthiocholine iodide as substrate and 5,5' dithio-(bis-2-nitrobenzoic acid) (DTNB) as a coupler. Briefly, 0.125 U AChE was incubated in 1 ml of 0.1 M phosphate buffer (pH 8.0) containing 0.3 mM DTNB and test compounds for 2 min at 37°C. The reaction was started by addition of 0.5 mM acetylthiocholine and activity measured spectrophotometrically at 412 nm as a function of time with Beckman (Fullerton, CA, USA) DU-650 spectrophotometer. All operations were performed in the dark due to the photosensitivity of the AChE and the PAHs. The Km value for AChE under the assay condition used was found to be 0.3±0.02 mM.

RESULTS

Inhibition of Acetylcholinesterase by Polycyclic Aromatic Hydrocarbons

The effect of polycyclic aromatic hydrocarbons (PAHs) on the activity of acetylcholinesterase (AChE)

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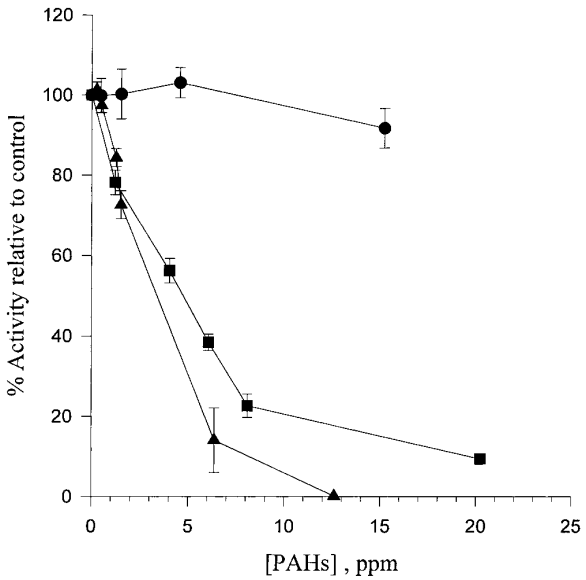

FIG. 1. Effect of polycyclic aromatic hydrocarbon compounds on the acetylcholinesterase activity *in vitro*. The activity of acetylcholinesterase purified from electric eel were assayed according to procedure outlined under Methods. The polycyclic aromatic hydrocarbons (PAHs) including benzo[a]pyrene (▲), pyrene (■) and acenaphthylene (●) were added to the reaction buffer at various concentration indicated and incubated for 5 min before activity measurements. The percent activity was calculated relative to the activity with solvent used to dissolve the PAHs. The data were expressed as mean ± S.E.M. (n⩾3).

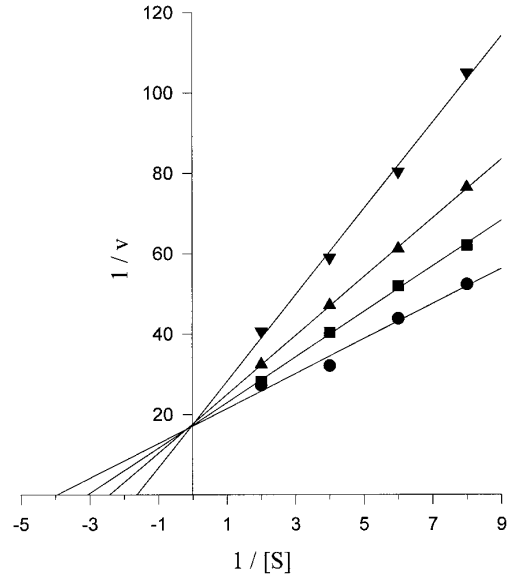

FIG. 2. Double reciprocal Lineweaver-burk plot of benzo[a]-pyrene on the acetylcholinesterase activity *in vitro*. The activity of acetylcholinesterase purified from electric eel were assayed . The acetylcholinesterase was incubated in the absence of and presence of (●), 6 μM (■), 8 μM (▲) and 10 μM (▼) of benzo[a]pyrene in 1 ml of assay buffer for 5 min and activity measured according to procedure outlined under Methods with different concentrations of substrate. The data expressed were the mean value from three experiments with triplicate determinants.

purified from electric eel were determined. As shown in Figure 1, benzo[a]pyrene and pyrene dose-dependently inhibited the activity of AChE with IC_{50} values of 2.67±0.341 ppm and 5.22±0.38 ppm, respectively. In contrast to benzo[a]pyrene, acenaphthylene showed only slight inhibition of the AChE activity at concentrations up to 15.2 ppm. Higher concentrations of acenaphthylene were not tested due to the solubility problem. The effect of other PAHs on AChE were also examined and the IC_{50} values were summarized in Table 1. PAHs with 3 or higher aromatic rings all showed inhibitory effect on the AChE; however, the PAHs with lower number of aromatic rings, such as naphthalene and fluorene and the oxygenated PAHs, such as xanthone and anthraquinone, as seen with acenaphthylene (Figure 1) have little effect on the activity of AChE.

TABLE 1

IC_{50} Values of Polycyclic Aromatic Hydrocarbons on Acetylcholinesterase Activity

PAHs	IC_{50} (ppm)
Anthracene	3.85 ± 0.42
Benzo[a]pyrene	2.67 ± 0.34
Chrysene	2.40 ± 0.04
Fluoranthene	4.68 ± 0.21
Nitropyrene	3.05 ± 0.40
Pyrene	5.22 ± 0.38

Note. The inhibitory effect of polycyclic aromatic hydrocarbons at various concentrations were examined as stated in Methods and IC_{50} values calculated. The data were expressed as mean ± S.E.M. (n⩾3).

Polycyclic Aromatic Hydrocarbons Competitively Inhibited Acetylcholinesterase Activity

In order to study the mechanism of this inhibitory effect seen with PAHs on AChE, the kinetic data were analyzed with the double reciprocal Lineweaver-burk plot (Figure 2). We have found that benzo[a]pyrene competitively inhibited the AChE with Ki value of 3.50±0.29 ppm. Other PAHs, which inhibited the AChE, also act as competitive inhibitor of AChE and the K_i values were summarized in Table 2.

DISCUSSION

Organic contaminants of most concern tend to be those that are environmentally stable such as polycyclic aromatic hydrocarbons which originate from a number of sources and, due to their stability, tend to have become ubiquitous food contaminants which accumulate particularly in fatty foods. Of particular environmental concern are PAHs ranging in molecular weight from 128 (naphthalene) to 300 (coronene), PAH

TABLE 2

K_i Values of Polycyclic Aromatic Hydrocarbons on Acetylcholinesterase Activity

PAHs	K_i (ppm)
Anthracene	3.80 ± 0.32
Benzo[a]pyrene	3.50 ± 0.29
Chrysene	2.67 ± 0.07
Fluoranthene	4.96 ± 0.07
Nitropyrene	2.63 ± 0.55
Pyrene	6.57 ± 0.80

Note. The acetylcholinesterase activity at different concentrations of substrate and inhibitors were measured according to procedure outlined under Methods. The kinetic data were analyzed with the double reciprocal Lineweaver-burk plot and K_i values were calculated with the equation $-1/K_{mapp} = -1/K_m(1 \pm [I]/K_i)$. The data were expressed as mean ± S.E.M. ($n \geqslant 3$).

within this group tend to be more water soluble (10), increasing their potential bioavailability and mobility within the environment. Human exposure to PAHs is virtually unavoidable (11) and can occur through many routes, including the breathing of contaminated air, eating or drinking contaminated food or water and by smoking (12). Previous work on PAHs was mostly concentrated in their mutagenic and carcinogenic effects and immunotoxicity. Only a limited number of experimental studies have been conducted to assess the toxic effects of PAHs on other physiological functions, for example, the cardiovascular (13) and reproductive (14) systems.

In this study, we have shown that, for the first time, PAHs dose-dependently inhibited the AChE activity in a competitive manner. The PAHs containing 3 or higher aromatic rings showed the inhibitory effect with the chrysene being the most potent inhibitor based on the IC_{50} and K_i values. PAHs with lower number of aromatic rings, such as naphthalene, acenaphthylene and fluorene, and oxygenated PAHs, such as anthraquinone and xanthone, showed no or slight inhibition of AChE activity.

ACh is one of the most important neurotransmitter in either central or peripheral nervous system and the inhibition of AChE has been proposed as biomarker for the neurotoxicity (15). In addition, Sagai *et al.* (16) have recently shown that *in vivo* administration of the extract of diesel exhaust particle (DEP) increased the airway hyper-responsiveness to ACh. Because of their low vapor pressures, PAHs are bound to airborne particles, such as the DEP (17), to a high degree (18). However, the possible correlation with the anti-AChE effect by PAHs observed in this study to their findings still await for further investigation in the future.

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