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Distribution of Benzo(a)pyrene in Discrete Regions of Rat Brain

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Benzo(a)pyrene (BP), a polycyclic aromatic hydrocarbon (PAH) is a ubiquitous environmental pollutant and a potent carcinogen (Heidelberger 1975). The factors responsible for tumorigenesis of BP are multiple and complex. It is generally accepted that BP is metabolically activated by one or several enzymes to reactive electrophilic intermediates which are capable of binding to nucleophiles (Weinstein et al 1978; Brooks et al 1978). The metabolism of BP is carried out by a cytochrome P-450 (P-450) dependent mono-oxygenase system commonly known as aryl hydrocarbon hydroxylase (AHH) and a non P-450 dependent enzyme, epoxide hydrolase (Heidelberger 1975; DePierre and Ernster 1978). While BP metabolism in liver has been extensively studied relatively little work has been performed in extraheaptic tissues such as brain. Juchau et al (1979) have shown that brain of fetal and adult mice and rats converts PAH(s) including BP to mutagenic and cytotoxic metabolites. Our studies have also focussed on characterization of AHH activity in brain microsomes and mitochondria (Das et al 1981a; 1981b; 1982a). In this communication we report the distribution and retention of radiolabelled BP in discrete regions of rat brain.

MATERIALS AND METHODS

[^{3}H]BP (4.1 Ci/mM) radiochemical purity 99%, was a product of Bhabha Atomic Research Centre, Bombay, India. [^{3}H]BP was suspended in peanut oil at a concentration of 120 uCi/ml. Male wistar albino rats (110 ± 10 g) derived from the Industrial Toxicology Research Centre, Lucknow, animal breeding colony were given a single intraperitoneal injection of 60 uCi (15 nmole) of [^{3}H]BP. The rats were sacrificed at 1,3,5,8,18,24,48,72 and 96 hours after the administration of radiolabelled BP. The desired tissues were removed from the body, rinsed three times in 0.1 M phosphate buffer (pH 7.4), and a portion (35-45 mg) of tissues or brain region was solubilized in 0.8 ml of hydroxide of hyamine, a product of Packard Co., USA. A 0.4 ml aliquot of solubilized tissue suspension was taken in scintillation vial to which 10 ml of scintillation fluid was added (toluene 250 ml, dioxane 250 ml, methanol 150 ml, naphthalene 52 g, 2,5-diphenyl oxazole (PPO) 3.25 g, 1,4-bis-2 (5-phenyl oxazolyl)benzene (POPOP) 0.65 g). The radioactivity was determined in a Packard Liquid Scintillation Counter with a counting efficiency of 48%.

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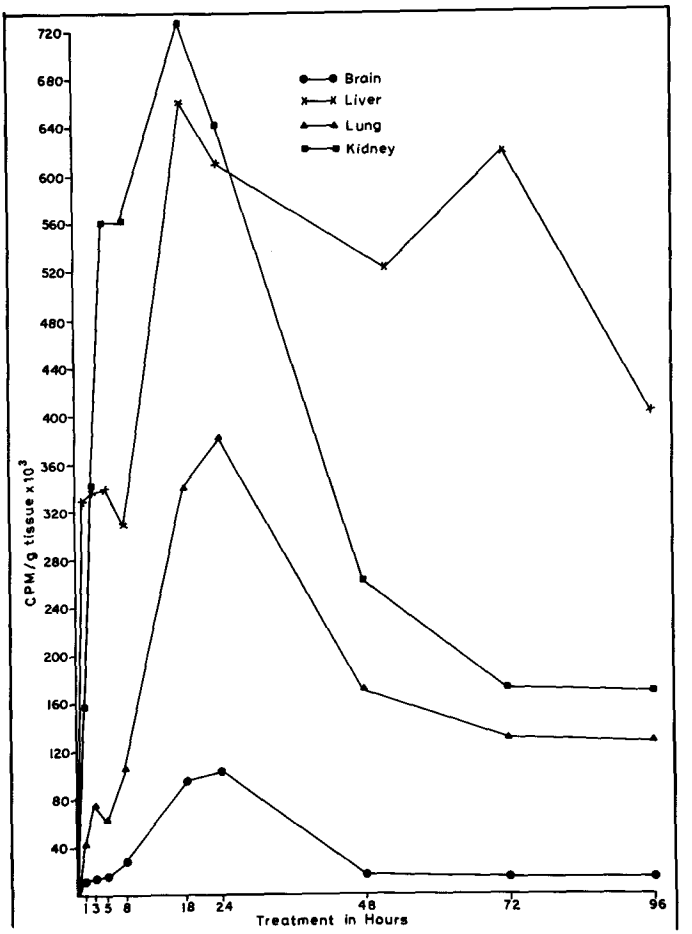

Figure 1. Distribution of [^{3}H]benzo(a)pyrene in different organs of rat. Rats were treated with [^{3}H]BP as described under "Materials and Methods". Data represent mean of four rats with a variation of 10%.

RESULTS AND DISCUSSION

The distribution of radiolabelled BP in different rat organs is shown in figure 1. It is evident that even after 1 hour of intraperitoneal administration of [^{3}H]BP significant radioactivity was detected in brain. It attains maximum concentrations at 24 hours of [^{3}H]BP treatment followed by a rapid decline at subsequent periods. BP is rapidly diffused into liver and acquires peak concentration at 18 hours. The concentration of BP in lung and kidney showed the peak at 24 and 18 hours respectively. The biokinetic profile of BP in different tissues analyzed followed the order: liver kidney lung brain (Fig. 1).

Table 1. Distribution of [^{3}H]benzo(a)pyrene in discrete regions of rat brain

Treatment (hours)	Cerebellum	Medulla & pons	Corpus striatum	Hypothalaumus	Mid brain	Hippocampus	Cortex	Olfactory lobes
1	15.0 ± 0.6	11.3 ± 0.6	15.1 ± 0.4	14.0 ± 0.6	15.4 ± 0.4	17.4 ± 0.8	15.7 ± 0.5	17.9 ± 0.7
3	15.7 ± 0.8	12.7 ± 0.6	15.6 ± 0.1	14.2 ± 0.3	15.9 ± 0.5	19.1 ± 0.5	16.2 ± 0.7	20.0 ± 0.7
5	14.1 ± 0.5	15.2 ± 0.2	15.2 ± 0.5	14.7 ± 0.7	14.4 ± 1.4	19.9 ± 1.5	16.4 ± 1.4	21.8 ± 0.6
8	37.0 ± 1.2	39.8 ± 2.8	36.8 ± 3.1	34.6 ± 2.7	31.8 ± 2.4	30.7 ± 1.3	30.1 ± 1.2	48.5 ± 1.1
18	37.9 ± 1.2	30.0 ± 1.0	32.7 ± 1.3	36.6 ± 3.2	31.4 ± 1.3	37.5 ± 1.1	36.2 ± 1.2	60.9 ± 2.6
24	98.3 ± 0.7	70.4 ± 6.0	74.4 ± 2.6	64.5 ± 2.3	76.2 ± 4.7	68.9 ± 2.2	66.4 ± 4.1	109.8 ± 5.1
48	13.6 ± 0.7	15.8 ± 0.7	13.4 ± 0.6	14.7 ± 0.6	13.9 ± 0.8	15.1 ± 0.8	14.1 ± 1.1	19.6 ± 2.1
72	10.9 ± 0.9	12.2 ± 1.1	13.5 ± 0.8	11.2 ± 0.6	12.0 ± 0.9	11.0 ± 1.4	12.1 ± 0.9	11.9 ± 0.9
96	11.5 ± 0.8	12.4 ± 0.6	14.0 ± 0.7	12.8 ± 0.4	10.7 ± 0.8	12.5 ± 0.9	11.4 ± 0.8	12.5 ± 1.0

Treatment was done as described under "Materials and Methods".

Data represent mean ± S.E. of four values.

Radioactivity was determined and calculated as CPM x 10^3/g brain region. Radioactive counts shown in this study were accounted for quenching.

The distribution of [^{3}H]BP in different brain regions is delineated in table 1. All the regions showed a peak concentration of radioactivity at 24 hours after [^{3}H]BP treatment. The maximum concentration of [^{3}H]BP (CPM/g wt) at 24 hours in the brain regions followed the order: olfactory lobes cerebellum mid brain corpus striatum medulla & pons hippocampus cortex hypothalamus (Table 1).

The present study suggests that BP and/or its metabolites are significantly distributed in all the regions of rat brain. The radioactivity in different brain regions may be due to the metabolism at the target site or due to the metabolism at other site such as liver passing through the blood brain barrier. Since all the regions of brain possess AHH activity (Das et al 1982a; 1982b), BP entering into them would be expected to be metabolized. Among the brain regions the AHH activity is high in olfactory lobes and cerebellum (Das et al 1982b) and the present study shows that radioactivity in the same regions are also high suggesting a greater accumulation of BP and/or its metabolites in these regions.

The studies on the metabolic profile of BP in control and 5,6-benzoflavone stimulated fetal and adult rat brain microsomes have shown that BP is metabolized to trans-9, 10-diol, trans-4,5-diol, trans-7,8-diol, quinones, 3-OH and 9-OH metabolites (Rouet et al 1981). The secondary metabolism of some of these metabolites may bind to macromolecules to elicit its carcinogenic effects. Thus, the organisms living in a hostile PAH environment may be more susceptible towards transplacental brain tumors as observed by 3-methylcholanthrene and 7,12-dimethyl benz(a)anthracene (Zimmerman and Arnold 1941; Rice et al 1978).

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