EFFECT OF BENZ(a)PYRENE AND ITS NONCARCINOGENIC ANALOG 1,2-BENZPYRENE ON Na,K-ATPase ACTIVITY OF MOUSE ORGAN HOMOGENATES AND A RAT HEART MEMBRANE PREPARATION

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The first structure with which a chemical carcinogen interacts in the cell is the membrane [1]. The membrane protein which has been studied the most is Na,K-ATPase, whose function depends on the state of the cell membrane. The action of chemical carcinogens on Na,K-ATPase has received little study although it has been shown that its activity and regulatory properties are modified during carcinogenesis [2, 5].

The effect of the carcinogen benz(a)pyrene (BP) and its noncarcinogenic analog 1,2-benzpyrene (1,2-BP) on Na,K-ATPase activity of mouse lung, forestomach, and liver homogenates and on a membrane preparation of rat heart was studied in the present investigation.

EXPERIMENTAL METHOD

Female albino mice weighing 20-30 g were used in the experiments during the summer. After decapitation of the mouse the isolated organs were placed in cold physiological saline (0.9% NaCl), then weighed and ground at 0-4°C in a glass homogenizer in the proportion of 1 g tissue to 20 ml of solution. The membrane preparation of Na,K-ATPase from rat myocardium was obtained by a modified method of Dietz and Hepp [3]. ATPase activity was determined from the rate of accumulation of inorganic phosphate (P_i). BP and 1,2-BP were dissolved in ethanol (their final concentration in the incubation medium was 10^{-7} M). Samples containing 2 mM ethanol were used as the control. Total ATPase activity was determined in incubation medium of the following composition: ATP — 2 mM, $MgCl_2$ — 2 mM, NaCl — 130 mM, KCl — 30 mM, Tris-HCl buffer, 50 mM, pH 7.4. Mg-ATPase was determined in the same medium with the addition of ouabain (1 mM) or in an incubation medium containing 2 mM ATP, 2 mM $MgCl_2$, 160 mM NaCl, 50 mM Tris-HCl buffer, pH 7.4. The Na,K-ATPase activity of the homogenate was taken to be the difference between activities of total and Mg-ATPase. The Na,K-ATPase activity of the membrane preparation from rat heart was determined in a medium of the following composition (mM): ATP — 3 mM, $MgCl_2$ — 5 mM, NaCl — 100 mM, KCl — 20 mM, ethyleneglycol tetraacetate (EGTA) 1 mM, Tris-HCl buffer, 50 mM, pH 7.4. The incubation time was 10 min, the volume of the sample 1 ml, and the temperature 37°C.

The reaction was started by addition of the membrane preparation (60 μg) or homogenate (100-150 μg protein) and stopped by the addition of TCA. The P_i concentration was determined colorimetrically [4] and protein by the biuret method. The sources of the reagents were as follows: sodium deoxycholate, Tris-HCl, and ATP were from Reanal, Hungary, EGTA from Lamont Lab., USA, ouabain from Merck, West Germany, and BP and 1,2-BP from Fluka A. G., Switzerland. The remaining reagents were from Soyuzreaktiv, USSR, and were of the chemically pure grade.

The results were subjected to statistical analysis by means of a nonparametric criterion of signs.

EXPERIMENTAL RESULTS

The total number of experiments was 30. Their results are given in Table 1.

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TABLE 1. Effect of BP and Its Noncarcinogenic Analogs 1,2-BP on Na,K-ATPase Activity (in μmoles P_i/mg protein/h) of Various Mouse Organ Homogenates and a Rat Heart Membrane Preparation of Na,K-ATPase

Experimental conditions	Homogenates of organs			Rat heart membrane preparation of Na,K-ATPase
	forestomach	liver	lungs	
Control	0,49 (0,10—1,15)	0,05 (0,03—0,07)	1,72 (0,82—4,60)	3,60 (3,00—5,00)
1,2-BP	0,20 (0,0—0,98)	0,04 (0,0—0,11)	0,94 (0,22—3,72)	5,40 (4,90—5,90)
P	<0,05	>0,05	<0,05	>0,05
BP	0,08 (0,0—0,22)	0,05 (0,0—0,17)	0,0 (0,0—0,0)	4,75 (3,70—5,80)
P	<0,05	>0,05	<0,05	>0,05

Legend. Limits of variations shown in parentheses.

The carcinogen BP significantly reduced Na,K-ATPase activity in homogenates of the lungs and forestomach from 1.72 and 0.49 μmole P_i/mg protein/h to zero and 0.08 μmole P_i/mg protein/h respectively.

The action of 1,2-BP on these organs was less marked. In lung homogenates Na,K-ATPase activity was inhibited by 46%, and in the homogenate of the forestomach by 59%. Neither compound had any significant action on Na,K-ATPase activity of the liver homogenate or rat myocardial membrane preparation of Na,K-ATPase.

The two compounds were thus effective only against homogenates of the lungs and forestomach; BP inhibited the enzyme more strongly than 1,2-BP. This result is interesting, because BP can induce malignant neoplasms in these organs, and indeed they are its target organs. Meanwhile the liver and heart are not target organs for BP, and this correlates with the absence of effect of BP on their Na,K-ATPase activity. The results are in good agreement with views on the important role of Na,K-ATPase in carcinogenesis .

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