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SIMILAR LEVEL OF METABOLIC ACTIVATION OF BENZO(A)PYRENE IN PERFUSED RAT LUNG AND LIVER AND PROTECTION OF LUNG BY LIVER IN A COMBINED PERFUSION SYSTEM

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SUMMARY. Irreversible binding of metabolically activated benzo(a)pyrene to DNA, RNA and protein proceeds by a different time course in perfused liver and lung of 5,6-benzoflavone-treated rats. Peak binding in liver is obtained after 15 min while binding in lung continuously increases over 120 min. Total irreversible binding per mg DNA or RNA is in the same order of magnitude in both organs. While binding in lung is lower at 15 min it exceeds binding in liver at 120 min. Binding per mg protein is higher in lung than in liver over the whole perfusion period. Introduction of a liver into the lung perfusion circuit decreases binding in lung. This protection effect is more pronounced when the liver is obtained from an animal pretreated with 5,6-benzoflavone.

The liver is the tissue which has by far the highest capacity to metabolize foreign compounds including chemical carcinogens. This is also true for such carcinogens for which the liver is not a target organ, e.g. benzo(a)pyrene. In lung microsomes benzo(a)pyrene metabolism proceeds by a rate which is only 1 % or less of that in liver microsomes (1,2), and a similar relation is also found in perfused organs (3). Irreversible binding of metabolically activated benzo-(a)pyrene has been measured in perfused rat liver and lung (4-7), but a comparison based on identical experimental conditions has not yet been performed. It may be assumed that the higher formation rate of active intermediates in the liver will also lead to higher levels of nucleic acid and protein modification by these intermediates. Moreover, one should suspect that once a carcinogen has become systemically available the liver will influence the amount of active metabolites irreversibly bound to cellular macromolecules in other tissues, f.i.

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the lung. On one hand, the liver may function as a detoxication site by its ability to remove the carcinogen from the circulation. On the other hand it is conceivable that the liver releases metabolites into the circulation which are taken up by the target organs and are reactivated there to provide binding material after conjugate cleavage. The present study was undertaken to compare benzo(a)pyrene metabolite binding to rat liver and lung and to test in a combined perfusion system the influence of the liver on the level of nucleic acid and protein modification by benzo(a)pyrene in the lung.

METHODS

Male Sprague-Dawley rats were pretreated with 80 mg/kg 5,6-benzoflavone dissolved in peanut oil 36 h before the experiment. Control animals received peanut oil. For perfusion the organs remained *in situ*. Liver perfusion was performed by the technique of Miller et al. (8) as modified by Hems et al. (9). The lungs were perfused via a cannula inserted into the truncus of the pulmonal arteries. The outflowing medium left the heart by an incision in the left ventricle and was collected by a funnel to return to the reservoir of the recirculating system. Ventilation was performed by a Starling pump with 15 x 1.2 ml air/min. The perfusion medium for all types of experiments consisted of 40 % washed bovine erythrocytes in Krebs-Ringer bicarbonate buffer, containing 0.75 g bovine serum albumin and 50 mg glucose in a final volume of 50 ml, and was gassed with 5 % CO_2 in O_2. The final pH was 7.4.

Generally labelled tritiated benzo(a)pyrene obtained from Amersham Buchler, Braunschweig, FRG, was used as the substrate in a concentration of 1 µM (about 1 mCi per perfusion). The substrate was washed before use with 0.25 n NaOH in 40 % ethanol and was shown to be chromatographically pure as judged from thin layer chromatography on silica gel (benzene : ethanol 90 : 10). Addition of substrate was performed after a 20-30 min equilibration phase in 0.2 ml ethanol. Protein and nucleic acid were isolated by the method of Diamond et al. (10) as modified by Kuroki and Heidelberger (11) and described in detail in a previous publication from this laboratory (4). DNA and RNA were essentially free of protein. After extensive washing, aliquots of the DNA, RNA and protein fractions were assayed for bound radioactivity by liquid scintillation counting. DNA was digested with DNase prior to counting.

RESULTS AND DISCUSSION

Time course of binding

Fig. 1 compares the time course of irreversible binding of metabolically activated benzo(a)pyrene to nucleic acids and protein of perfused liver and lung of 5,6-benzoflavone pretreated rats. In the liver a peak concentration of bound radioactivity is noticed at 15 min which then gradually declines. In contrast, binding in the lung continuously increases during the 2 h perfusion period. The reason for this organ difference is not obvious. One may speculate that the liver by its high detoxication capacity is more efficient to remove unmetabolized benzo(a)pyrene from the medium so that substrate availability becomes limi-

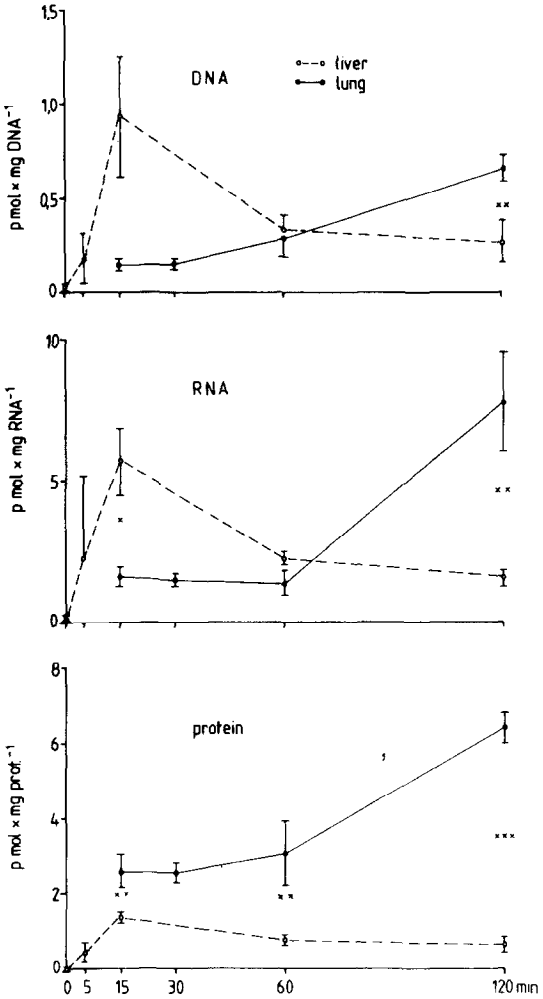

Fig. 1 Time course of total irreversible binding of metabolically activated benzo(a)pyrene to nucleic acids and protein of perfused rat liver and lung. Values are means ± S.E. (n = 3-7). * $p < 0.05$, ** $p < 0.002$, *** $p < 0.001$ (liver versus lung). Organs were obtained from animals pretreated with 5,6-benzoflavone.

ting after 15 min of perfusion. However, even at the low substrate concentration of 1 µM used in our experiments we find relatively high concentrations of unmetabolized benzo(a)pyrene in the liver perfusion fluid up to 60 min. It cannot be excluded that in spite of extensive washing of the isolated macromolecules free or loosely bound benzo(a)pyrene metabolites contribute to the binding values. Such material would be subject to the potent detoxifying processes in liver during the perfusion period thus mimicking loss of irreversible binding. Further, it cannot be excluded that processes leading to removal of adducts act much more rapidly in liver than in lung. Repair processes are well known for

DNA, and it is conceivable that they start as early as during the first hour after adduct formation. Removal of chemically modified RNA and protein is less well characterized, and it is not yet known if modification leads to more rapid turnover of these molecules.

Comparison of total binding in liver and lung

The extent of binding is comparable in liver and lung of animals in which cytochrome P-448 had been induced. While at 15 min binding to nucleic acids of liver exceeds that to nucleic acids of lung by a factor of 3-6, the relation is nearly reversed after 120 min of perfusion. When maximal binding as measured in our experiments (at 15 min in liver and at 120 min in lung) is compared, very similar values for DNA and RNA are obtained. Protein binding is far more pronounced in lung than in liver at all time points. It should be noted that peak binding in lung has not been determined and may be higher than binding at 120 min. The amount of total binding to lung DNA is in the same order of magnitude as that found by Vähäkangas et al. (7) at similar substrate concentration. Total binding in perfused liver at such low substrate concentrations has not been reported previously.

These results are in contrast to what should have been expected from the large differences in benzo(a)pyrene-metabolizing capacity of liver and lung as measured in microsomal preparations of rat (2) and mouse (1) at saturating substrate concentrations. That these differences are still such pronounced at low substrate concentrations is suggested by perfusion experiments of Vähäkangas et al.(3). Induction of cytochrome P-448 - as performed in our experiments - slightly decreases these differences but even in this situation they are still very prominent (12) Nevertheless, similarly as to what our perfusion experiments have shown, comparable activation rates of a benzo(a)pyrene metabolite (3-hydroxybenzo(a)pyrene) by rat liver and lung microsomes have been demonstrated (13). It appears that monooxygenase activity is not the main limiting factor in adduct formation with benzo(a)pyrene metabolites. It is not yet clear which other factors are involved. One may be differences in metabolite pattern in both organs: The percentage of dihydrodiols formed is higher in lung than in liver (1,2), perhaps because of a

higher relation of epoxide hydrolase to monooxygenase activity in lung (1). Another factor may be the differences in detoxication capacity of both organs. It is conceivable that the liver is able to rapidly remove the large amount of reactive material once formed while the lung with its lower conjugation capacity will not be able to deal so well with the lower amounts of active metabolites it forms Biliary excretion may contribute to this relation.

In order to interpret the biological consequences of the identical amount of binding in both organs as well as the longer persistence of binding in lung, it seems necessary to determine possible differences in the velocity of the removal of individual adducts. The ultimate carcinogen derived from benzo(a)pyrene is assumed to be the 7,8-dihydrodiol-9,10-epoxide (14), and this adduct has been detected both in perfused liver (4,5) and lung (6,7). In addition several other adducts are found in perfused liver, including an adduct with the 9-hydroxy-4,5-oxide (5) which has also been detected in perfused lung (6). There is evidence that the critical diol epoxide adduct is removed more slowly than the phenol oxide adduct in hepatocytes (15) and perfused liver (16). Without substantiation of these findings the conclusion which may be drawn from our results remains only preliminary that the higher persistence of irreversible binding in lung may contribute - in addition to other factors, f.i. that lung is a primary exposition site to polycyclic hydrocarbons entering the body via inhalation - to the fact that lung but not liver is a target organ of benzo(a)pyrene carcinogenesis.

Influence of the liver on binding to lung macromolecules

In Fig. 2 the influence of a liver introduced into the perfusion circuit of the 5,6-benzoflavone-induced lung is demonstrated. Total binding is reduced by this procedure, and this effect is far more pronounced when the liver is obtained from an animal in which cytochrome P-448 had also been induced. The data show that the high detoxication capacity of the liver can protect other organs having lower enzymic capacity in dealing with active metabolites against systemically available benzo(a)pyrene; they do, however, not exclude that some reactive material released by the liver in conjugated form may be taken up and reactivated by the lung. Such effect, if it exists, is readily overcome by the clearing function

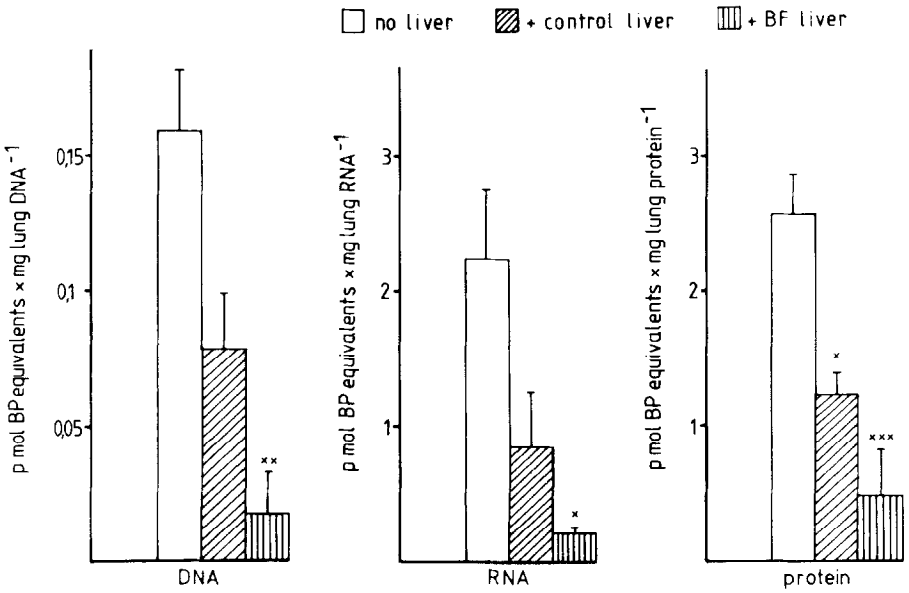

Fig. 2 Influence of the insertion of a liver into the lung perfusion circuit on total irreversible binding of metabolically activated benzo(a)-pyrene to pulmonary nucleic acids and protein. Values are means ± S.E. (n = 3-7). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ versus no liver-values. The lungs were obtained from animals pretreated with 5,6-benzo-flavone. Livers were either obtained from oil-treated animals (control liver) or from 5,6-benzoflavone-pretreated animals (BF liver). Perfusion time was 30 min.

of the liver. Experiments in which the perfusion medium of a liver exposed to benzo(a)pyrene is subsequently used for lung perfusion should help evaluating this question. It is likely that the clearing effect is due to removal of unmeta bolized benzo(a)pyrene from the circulation. One should keep in mind that the perfusion model used does not exactly mimick the anatomical situation *in vivo*; the distance between liver and lung is much smaller in the intact rat than in in the perfusion system, and the flow rate is much higher *in vivo*. The inter-action of liver and lung described here may be of consequence for pulmonary bin-ding of systemically available carcinogens in individuals with impaired liver function. Moreover, the data suggest that the high inducibility of lung monooxy-genase is of little importance for lung macromolecule binding in a metabolic situation in which liver monooxygenase is also induced. This is supported by preliminary data showing similar levels of irreversible binding in noninduced lung in the presence of an noninduced liver (DNA: 0.02 ± 0.00, RNA: 0.21 ± 0.04, protein: 0.16 ± 0.03 pmol x mg^{-1}) as in induced lung in the presence of an in-

duced liver (Data of Fig. 2). However, in a situation in which lung enzymes are selectively induced, f.i. by inhalation of inducers, high carcinogen binding to lung macromolecules as expected from the high inducibility of pulmonary monooxygenase and as substantiated by our perfusion experiments with induced lungs in the absence of a liver may indeed occur.

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