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EIGHTFOLD INDUCTION OF NICOTINE ELIMINATION IN PERFUSED RAT LIVER BY PRETREATMENT WITH PHENOBARBITAL

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SUMMARY. Elimination of nicotine by isolated rat livers was increased eightfold after pretreatment with phenobarbital (PB) as an inducer of cytochrome P-450, while it was only marginally influenced after pretreatment with 5,6-benzoflavone (BF) as an inducer of cytochrome P-448. Initial rates of cotinine formation were enhanced in the same order of magnitude in PB-induced livers. The ^{14}C-nicotine-derived radioactivity excreted into bile within 2 h ranged between 6 - 17 % of the dose with only 2.7fold higher values after PB pretreatment compared to controls.

Nicotine which is quantitatively the most important component in tobacco smoke is considered to reach the systemic circulation mainly by absorption from lung epithelium. The plasma concentrations of nicotine after occasional exposure to single cigarettes rapidly decline by distribution processes and metabolic transformation (for review see 1, 2). Hepatic monooxygenase activity is supposed to exhibit the highest capacity to metabolize nicotine (2, 3, 4). A varity of nicotine metabolites has been observed as products of microsomal oxidation, e.g. N-oxides, 5-hydroxynicotine and nicotine iminium ion as precursors of cotinine, as well as demethylation products of nicotine and cotinine (1, 2). Recently, 4-hydroxycotinine which had been described as a secondary metabolite of administered cotinine in animal species (5) was also detected

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in the urine of smokers as a significant metabolite (also termed 3-hydroxycotinine, 6). After primary oxidation steps mediated by the PB-inducible form of cytochrome P-450 (7, 8) aldehyde dehydrogenases were shown to be necessary for the final transformation to cotinine (9, 10). The aim of the present study was to evaluate the effect of inducers of different forms of cytochrome P-450 on the level of the intact organ using the *semi-in vivo* system of an isolated perfused liver.

METHODS

Male Sprague-Dawley rats (260-290 g) were pretreated with 3 x 80 mg/kg phenobarbital (PB) for 3 days or with 80 mg/kg 5,6-benzoflavone (BF) 48 h before the experiment. The respective control treatments were 3.3 ml/kg saline or 4 ml/kg peanut oil. Liver perfusion was performed by the technique of Miller et al. (11) as modified by Hems et al. (12) at a constant flow rate of 12 ml/min with the liver remaining *in situ*. The perfusion medium 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. After gassing with 5 % CO_2 in O_2 the final pH was 7.4.
Nicotine, (2-^{14}C-pyrrolidine nicotine, 1.9×10^9 Bq/mmol) was obtained from DuPont-NEN, Dreieich, F.R.G. After a 15 min equilibration period the substrate was administered in saline as a bolus of 25 nmol in order to establish an initial concentration of 0.5 µM. A 200 µl aliquot of the perfusate was made alkaline using 200 µl borate buffer (pH 9.2). After extraction with 200 µl of dichloromethane a 40 µl aliquot was directly applied onto F_{254} coated silicagel TLC plates. Chromatography was performed in chloroform/methanol (9:1, v/v). For quantitation the radiochromatograms were registrated using a TLC linear analyzer LB 284 (Berthold, Wildbad, F.R.G.).
The pharmacokinetic parameters were derived as follows: The elimination rate constant (k_β) was obtained by least squares regression analysis of the terminal phase of the concentration-time curve. The elimination half-life was calculated according to $(\ln 2)/k_\beta$. Areas under the concentration-time curve were determined by the trapezoidal method and extrapolated to infinity. Clearance (Cl) values were calculated by dose/AUC; the volume of distribution (V_d) was estimated as Cl/k_β. The values represent arithmetic means $\pm$ SE (n=4); statistical analysis was performed using student's t-test for independent samples (two-tailed).

RESULTS AND DISCUSSION

Fig. 1 compares the time course of nicotine concentrations in the perfusate of isolated livers perfused after pretreatment with PB, BF or after the respective control treatments. A very marked in-

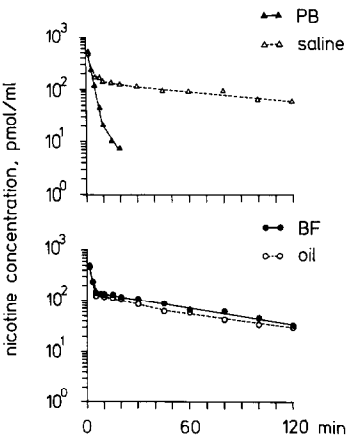

Fig. 1. Time course of nicotine concentration in the perfusate of isolated livers taken from PB, BF, or control-treated rats. Nicotine was administered as a bolus to give an initial concentration of 0.5 µM. Livers were perfused for 2 h using a recirculating perfusion system.

crease of the nicotine elimination rate was found after pretreatment with PB as an inducer of cytochrome P-450 whereas no influence of pretreatment with BF as an inducer of cytochrome P-448 was observed. Independently from the pretreatment schedule, the major part of the ^{14}C-nicotine-derived radioactivity remained in the perfusion medium within the 2 hours of perfusion. At the end of the perfusion 70 % (PB), 74 % (saline), 75 % (BF) and 78 % (peanut oil) of the total administered radioactivity was found in the medium. The respective pharmacokinetic parameters are given in Table 1. The unexpectedly high increase of the nicotine clearance is hardly due to gross changes in distribution processes as judged from the calculated volume of distribution (despite considerable variation) and from the comparably high amounts of radioactivity recovered in the medium after pretreatments.

On the other hand, the relatively low clearance values between 1.5 and 2.1 ml/min in isolated livers of uninduced or cytochrome P-448-induced rats point to a rather limited hepatic elimination capacity for nicotine, at least in rat. The extraction ratios which were calculated from the clearance values and the flow rates ran-

Table 1

Influence of pretreatment on the elimination of nicotine in isolated perfused rat liver

pretreatment	Phenobarbital	saline	5,6-benzoflavone	oil
$t_{1/2}$, min	5.6 ± 1.4***	82.8 ± 6.2	52.1 ± 7.0	73.2 ± 12.6
Cl, ml/min	11.6 ± 0.4***	1.5 ± 0.3	2.1 ± 0.2	1.7 ± 0.4
V_D, ml	110 ± 25	177 ± 40	157 ± 16	154 ± 18
E	0.93 ± 0.02***	0.12 ± 0.02	0.18 ± 0.02	0.13 ± 0.03
Cotinine formation pmol/min	522 ± 55***	54 ± 8	104 ± 15	69 ± 14
% of dose in bile	16.8 ± 1.6***	6.3 ± 1.0	11.8 ± 2.0	9.0 ± 3.4

Isolated rat livers from differentially pretreated rats were perfused with ^{14}C-nicotine (25 nmol = initial concentration 0.5 µM) dissolved in saline. A recirculating perfusion system was used at a flow rate of 12 ml/min.
The data represent arithmetic means ± SE (n=4); *** $p < 0.005$ induced versus uninduced. Metabolite formation rate was calculated from the linear phase of cotinine appearance in the perfusate. The value of biliary excretion is given as the amount of radioactive equivalents excreted within 2 hours.

ged between 0.12 and 0.18 for BF and control treatments, respectively. This means that more than 80 % of the dose administered in a submicromolar concentration range via the portal vein would escape first-pass metabolism in the liver. Our findings demonstrate that no flow limitation of nicotine elimination must be assumed under control and cytochrome P-448-induced conditions. This result is in contrast to what would have been expected from the physico-chemical properties of nicotine which would suggest high hepatic uptake and thus low systemic availabilty of nicotine after oral administration (13).

Pretreatment of rats with PB caused a marked increase of nicotine clearance which is now flow-limited as it can be seen from the calculated extraction ratio above 0.9 (Table 1). This effect of PB is probably mainly due to an increased metabolic clearance of nic-

otine because the calculated formation rate of cotinine as the main metabolite of nicotine was also induced approximately eight-fold. The values of metabolite formation given in Table 1 were calculated from the linear phase of cotinine release from the perfused organ into the medium. The maximum rate of metabolite formation may even be underestimated as secondary metabolism must be assumed from the decrease of cotinine concentrations in the perfusate after nicotine as well as after cotinine as the substrate (data not shown).

Within the 2 hours of perfusion, in the PB-stimulated liver about 17 % of the dose were excreted into the bile representing only a 2.7fold higher amount compared to the control value (Table 1). Thus, the biliary excretion of ^{14}C-nicotine-derived radioactivity showed a less prominent induction effect of PB than nicotine elimination from the perfusate. More than 90 % of the radioactivity in bile was not extractable into dichloromethane under alkaline conditions and, therefore, represent other substances than nicotine or cotinine as also judged from TLC analysis.

It is already well known that the initial step of nicotine oxidation is catalyzed by the PB-inducible form of cytochrome P-450 (7, 8) with *in vivo* Michaelis constants, depending on the species studied (14, 15), around 1 mM . Pretreatment of guinea pigs and mice with PB caused an induction of the metabolic transformation of nicotine to cotinine, but this effect did not exceed a factor of 2-3 when studied in mice *in vivo* (16) and was even lower when studied in incubations of guinea pig liver microsomes *in vitro* at a nicotine concentration of 0.5 mM (8). The products formed during the primary oxidation of nicotine are finally metabolized to cotinine by the activity of oxidoreductases (9). These enzymes which are located mainly in the cytosolic fraction are responsible for the secondary metabolism of nicotine iminium ion (10).

Pretreatment of rats with PB *in vivo* as well as treatment of cell cultures with PB *in vitro* also induces aldehyde dehydrogenases by a factor up to 15-20 depending on the substrate studied (17, 18, 19). The contribution of PB-induced oxidoreductases to the overall increase of nicotine metabolism as studied at low nicotine concentrations after pretreatment of rats with PB so far remains unanswered.

Regarding the nicotine plasma concentrations as an important factor for smoking behavior (20, 21), the consequences of a possibly marked diminuation of nicotine concentration after drug treatment leading to an induction of cytochrome P-450 activity in man remain to be evaluated. In addition, the suitability of cotinine plasma concentrations as a quantitative marker of smoking behavior might be questionable.

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