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HEPATIC BLOOD FLOW AND ENZYME INDUCTION IN THE RAT

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Abstract—Hepatic blood flow and hepatic BSP extraction were estimated in 11 rats injected with phenobarbital for four days and in 10 controls. Hepatic blood flow was not significantly different in phenobarbital-treated rats (8·42 ± 2·43 ml/min per 100 g body wt or 1·71 ± 0·42 ml/min per g liver, mean ± 1 S.D.) and in untreated rats (6·64 ± 2·76 ml/min per 100 g body wt or 2·04 ± 0·94 ml/min per g liver). In contrast, BSP extraction was significantly higher in phenobarbital-treated rats (0·83 ± 0·09) than in controls (0·69 ± 0·16; $P < 0·05$). These results suggest that the increase in BSP clearance observed in phenobarbital-treated rats results from increased hepatic BSP extraction and not from increased hepatic blood flow.

It is established that, in rats treated with phenobarbital, enzyme induction is accompanied by an increase in the sulfobromophthalein (BSP) plasma disappearance-rate constant [1, 2]. The aim of this study was to determine whether this increase is due to augmented BSP hepatic extraction or to augmented hepatic blood flow. If hepatic blood flow was augmented, this change in liver perfusion could contribute to the reduced plasma half-life of some drugs during enzyme induction.

MATERIALS AND METHODS

Adult male rats (Charles River, Saint-Aubin-lès-Elbeuf, France) weighing 300–400 g and fed *ad lib.* on UAR-113 biscuits (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France) were divided into two groups: (A) control animals; (B) rats injected intraperitoneally with increasing doses of sodium phenobarbital, viz. 8, 10, 12 and 14 mg of sodium phenobarbital per 100 g of body weight, on days 4, 3, 2 and 1, respectively, before the experiment.

The control group was subdivided into two subgroups: in subgroup A_1, the experiment was performed under the usual anaesthesia with pentobarbital (5 mg/100 g body wt); in subgroup A_2, the same dose of pentobarbital was injected, but the experiment was performed about one hour later, when the animals had awakened; these rats had to be restrained on the operating table and were much agitated.

The group B rats were anaesthetized with 5 mg/100 g body wt pentobarbital; owing to enzyme induction, the duration of anaesthesia was short in most animals so that they, too, were agitated throughout the experiment.

For the assessment of hepatic blood flow (HBF), the animals were immobilized on a heating table in order to maintain the rectal temperature at 38°C. Catheters were inserted into the left carotid artery and jugular vein. Hepatic vein catheterization was performed by means of a polyethylene catheter 0·7 mm o.d. (Biotrol No. 1, Paris, France), 12·5 cm long; the last centimeter was bent at a 50° angle; the last 2 mm were protected by a silastic sheath of 0·94 mm o.d. (available under No. 602-135 from Laboratoire Lepetit, Suresnes, France) designed to avoid perforating the vein wall. A metallic mandrel was inserted in the lumen of the catheter in order to keep it straight. The catheter with the mandrel was introduced into the right jugular vein and pushed through the right auricle into the inferior vena cava; stretching of the animal was necessary to permit the passsage of the catheter through the auricle; the catheter was pushed until 9 cm of its length was in the animal. The mandrel was then withdrawn and the catheter was gently pushed up and down until it met an elastic resistance, which corresponded to hepatic vein catheterization. A check on the positioning of the catheter can be provided by measuring the hepatic extraction of colloidal radiogold (30 μCi injected intravenously). Blood clotting was prevented by heparin.

After a loading dose, BSP was infused continuously into the left jugular vein for 30 min. The loading dose and the amount of BSP infused per minute were respectively 350 μg/100 g body wt and 75 μg/min per 100 g body wt in the controls and 750 μg/100 g body wt and 150 μg/min per 100 g body wt in the phenobarbital-treated rats. With these dosages, a plateau concentration of BSP was regularly obtained between 20 and 30 min after the beginning of the infusion. The dose of BSP was different in the two groups of animals for the following reasons: in preliminary experiments, when the dose of BSP used in the controls was administered to the phenobarbital-treated rats, dye

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concentration in hepatic venous blood was too low to be measured correctly; conversely, when BSP dosage used in phenobarbital-treated rats was administered to the controls, a plateau concentration could not be reached, BSP concentration in the plasma increasing constantly.

Blood samples, of 0·2 ml each, were taken every 2 min between 20 and 28 min, simultaneously from the carotid artery and the hepatic vein. After completion of the experiment, the animal was sacrificed, the position of the catheter was checked and the liver was immediately removed and weighed. BSP concentration in plasma was measured, with a spectrophotometer, at 580 nm after alkalization and appropriate dilution (50 μl of plasma were mixed with 20 μl of 3·50 M KOH and 200 μl 0·15 M phosphate buffer, pH 6·7). The haematocrit (Hct) was measured by a micromethod (CT 3400, Clay Adams, Parsipanny, NJ, U.S.A.). HBF was computed from BSP clearance (Cl) and BSP extraction (E), using the relation $HBF = (Cl/E)(1-Hct)$, where $Cl = Q/C_a$, Q being the quantity of BSP injected per min and C_a the plateau concentration of BSP in the plasma of the arterial blood, and where $E = (C_a-C_h)/C_a$, C_h being the plateau concentration of BSP in the plasma of the hepatic venous blood. The values for BSP plasma clearance were expressed in ml/min per 100 g body wt and the values for HBF in ml/min per 100 g body wt and in ml/min per g liver.

Cytochrome P-450 concentration was measured in liver homogenates [3] and the results were expressed as nmole of cytochrome P-450 per g liver. The means were compared using Student's *t*-test.

RESULTS

The values for BSP plasma clearance, BSP extraction and hepatic blood flow are set out in Table 1. BSP clearance and extraction were higher in group B than in group A. Liver weight and hepatic cytochrome P-450 content were respectively 3·23 ± 0·21 g/100 g body wt (mean ± 1 S.D.) and 36·93 ± 7·57 nmole/g liver in group A rats, and 4·86 ± 0·39 g/100 g body wt and 61·09 ± 11·26 nmole/g liver in group B animals. These means differ significantly ($P < 0·001$).

DISCUSSION

The increase in liver weight and the increase in cytochrome P-450 content, observed in the animals treated with phenobarbital, are regarded as valid indices of enzyme induction [4].

BSP clearance was significantly higher in the induced rats than in the controls: it must be emphasized that this increase in BSP clearance was observed although the amount of dye administered was higher in the induced animals than in the controls. The increase in BSP clearance should be viewed in relation to the increase in the BSP plasma disappearance-rate constant (or fractional clearance, or BSP K_1) measured after a single intravenous injection of this dye in phenobarbital-treated rats [2].

BSP extraction was significantly higher in the induced rats than in the controls. As with BSP clearance, the increase in BSP extraction is all the more significant as the dose of BSP infused was higher in the induced rats than in the controls; in the normal dog and in man, BSP extraction is reduced when the amount of dye is increased [5].

Hepatic blood flow was not significantly different in the induced rats and in the anaesthetized control animals. However the mean value for hepatic blood flow was slightly higher in the former than in the latter; that could be due to the fact that, in the induced rats receiving a standard dose of pentobarbital, anaesthetic deep sleep could not be obtained and that, therefore, these animals were often restless during the experiment; that unrest increases hepatic blood flow is supported by the finding that, in the control rats when awake, hepatic blood flow was significantly higher than in control rats under anaesthetic deep sleep. Our observation that hepatic blood flow is not significantly higher in induced rats than in control rats is at variance with the results of Ohnhaus *et al.* [6] who observed that hepatic

Table 1. BSP clearance, BSP extraction, and hepatic blood flow in control and in phenobarbital-treated rats

	BSP clearance (ml/min per 100 g body wt)	BSP extraction	Hepatic blood flow (ml/min per 100 g body wt)	Hepatic blood flow (ml/min per g liver)
(A_1) Anaesthetized control rats (N = 10)	2·29 ± 0·57	0·69 ± 0·16	6·64 ± 2·76	2·04 ± 0·94
(B) Phenobarbital-treated rats (N = 11)	3·98 ± 1·89†	0·83 ± 0·09*	8·42 ± 2·43‡	1·71 ± 0·42‡
(A_2) Unanaesthetized control rats (N = 8)	4·52 ± 0·71	0·73 ± 0·13	10·53 ± 1·83	2·72 ± 0·44

Results are expressed as means ± 1 S.D.
* Significantly different from anaesthetized control rats ($P < 0·05$).
† Significantly different from anaesthetized control rats ($P < 0·001$).
‡ Not significantly different from anaesthetized control rats.

blood flow increases by 33–175 per cent above the control value in rats treated with phenobarbital and with those of Branch *et al.* [7] who observed a 30 per cent increase in hepatic blood flow in monkeys receiving phenobarbital; however, it must be mentioned that, for measuring hepatic blood flow, these authors employed methods quite different from that used in the present work, either based on the principle of internal calorimetry [6] or on the regional distribution of cardiac output [7].

In conclusion, in phenobarbital-treated rats, BSP clearance is augmented as a consequence of increased dye extraction and not of increased hepatic blood flow.

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