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EFFECTS OF ALTERATION OF HEPATIC MICROSOMAL ENZYME ACTIVITY ON LIVER BLOOD FLOW IN THE RAT*

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Abstract—The effects of pretreatment with ten daily intraperitoneal injections of phenobarbital (8 mg/100 g/day) 3,4-benzpyrene (1 mg/100 g/day) and 3-methyl-cholanthrene (1 mg/100 g/day) and a single injection of SKF-525A (30 mg/kg) on liver blood flow in the rat were studied using radioactive microspheres. Of the hepatic enzyme-inducing agents only phenobarbital caused an increase in total liver blood flow, which averaged 33 per cent, and was associated with a similar increase in liver mass. The polycyclic hydrocarbons caused a small increase in liver mass of 11–15 per cent and no change in liver blood flow. All three agents induced drug-metabolizing enzymes, as judged by zoxazolamine paralysis time. SKF-525A produced no change in liver blood flow or liver mass. Liver blood flow/g of liver was unchanged in all groups. The increase in liver blood flow caused by phenobarbital was due entirely to an increase in flow to the splanchnic organs draining into the portal vein. These results imply that total hepatic blood flow is influenced by the mass of the liver and that the responsible, but as yet unknown, mechanism(s) involve the portal circulation. The different effect of phenobarbital and the polycyclic hydrocarbons on liver blood flow is only one of a number of distinctions between these inducing agents but may account for some of the differences observed *in vivo* especially with drugs whose clearance is dependent on liver blood flow.

The effect of drugs which alter hepatic enzyme activity on liver blood flow is controversial. Ohnhaus *et al.* [1, 2] found that induction of hepatic microsomal enzymes with phenobarbital and antipyrine but not benzpyrene resulted in increases in liver blood flow in the rat, whereas Denis *et al.* [3] could not demonstrate a significant change in hepatic blood flow with chronic phenobarbital treatment. In the rhesus monkey [4], we were able to show an increase in liver blood flow of 34 per cent with phenobarbital treatment. On the other hand, inhibition of hepatic enzyme activity in the rat with SKF-525A was reported to decrease colloidal gold clearance, which was interpreted to reflect a decrease in hepatic blood flow [5].

Much of the uncertainty in the rat seems to arise from technical difficulties in estimating blood flow to the liver. We have addressed this problem by adapting a radioactive microspheres method [6–8] for the measurement of organ blood flow and cardiac output in the rat, and have evaluated the effects on liver blood flow produced by enzyme induction with phenobarbital, 3,4-benzpyrene, 3-methylcholanthrene and enzyme inhibition with SKF-525A.

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METHODS

Enzyme induction. Groups of twelve male Sprague–Dawley rats, 250–350 g body weight (bw) (Harlan Industries, Indianapolis, Ind.), were given single daily injections intraperitoneally (i.p.) for 10 days. Sodium phenobarbital (8 mg/100 g, bw, in physiological saline) was injected in a volume of 1 ml/100 g, bw; 3,4-benzpyrene (1 mg/100 g, bw) and 3-methylcholanthrene (1 mg/100 g, bw) were administered in 0.5 ml corn oil/100 g, bw). Control groups received the same volumes of saline and corn oil. Fifteen to 20 hr after the last injection, one half of the animals were anesthetized with 35–45 mg/kg, bw of pentobarbital i.p. for hemodynamic studies and the other half were tested for zoxazolamine paralysis time.

Enzyme inhibition. Groups of thirteen male Sprague–Dawley rats, 250–350 g, bw, were given a single i.p. injection of either SKF-525A (30 mg/kg, bw) dissolved in physiological saline (20 mg/ml) or an equivalent volume of saline. Fifteen min later, the animals were anesthetized with 35 mg/kg, bw, of pentobarbital i.p., and hemodynamic studies were performed 40 min after the SKF-525A or saline injection.

Hemodynamic studies. Hemodynamic studies were performed by using $15 \pm 5\ \mu m$ microspheres labeled with ^{85}Sr. Polyethylene catheters were placed in the left ventricle via the right carotid artery and into the aorta via the right femoral vein. The microspheres were injected into the left ventricle in a volume of 0.8 ml warm 6% dextran over 20 sec with simultaneous withdrawal of a reference sample of arterial blood at 0.8 ml/min for 90 sec. Subsequently, the animals were killed with pentobarbital, and the liver,

Table 1. Effects of enzyme inducers on arterial pressure, cardiac output, liver weight and liver blood flow*

	Saline	Phenobarbital	Corn oil	3,4-Benzpyrene	3-Methyl-cholanthrene
Body wt (g)	337.5 ± 8.5	313.3 ± 12.8	309.0 ± 13.9	318.3 ± 9.5	300.8 ± 14.1
Mean arterial pressure (mm Hg)	126.0 ± 9.6	123.3 ± 4.4	121.9 ± 5.5	131.0 ± 4.1	124.0 ± 3.1
Cardiac output (ml/min/100 g, bw)	22.2 ± 0.8	24.8 ± 1.5	21.1 ± 2.3	20.5 ± 2.5	23.7 ± 2.9
Liver weight, (g)	12.0 ± 0.6	14.1 ± 0.2†	11.6 ± 0.7	13.1 ± 0.8	12.8 ± 0.6
Liver weight/body weight × 100	3.54 ± 0.10	5.05 ± 0.33†	3.69 ± 0.09	4.10 ± 0.17‡	4.27 ± 0.15†
Liver blood flow (ml/min/100 g, bw)	6.68 ± 0.23	8.86 ± 0.39†	6.61 ± 0.51	6.47 ± 0.81	6.72 ± 0.47
Liver blood flow (ml/min/g liver)	1.90 ± 0.09	1.83 ± 0.08	1.80 ± 0.14	1.58 ± 0.20	1.59 ± 0.13

* Means ± standard errors of groups of six rats.
† $P < 0.01$ as compared to appropriate control group (unpaired t-test).
‡ $P < 0.05$ as compared to appropriate control group (unpaired t-test).

spleen and the remainder of abdominal viscera were counted separately in a gamma scintillation counter. Cardiac output was calculated by multiplying the radioactivity injected by the reference sample withdrawal rate divided by the radioactivity in the reference sample. Organ blood flow was determined by multiplying the radioactivity in the organ by the reference sample withdrawal rate and dividing by the radioactivity in the reference sample. Since the microspheres are trapped on the first pass through a capillary bed, hepatic radioactivity represents only hepatic arterial flow. Portal venous flow is calculated from the radioactivity trapped in the organs draining into the portal vein. The advantages and problems with this technique have been discussed [6–8].

Zoxazolamine paralysis times were determined with 60 mg/kg, bw, of zoxazolamine i.p. Zoxazolamine (2 g) was dissolved in 24 ml of 1 N HCl and the solution diluted to 100 ml with 0.9% NaCl. If paralysis did not occur with 60 mg/kg, bw, up to two additional 20 mg/kg, bw doses were given at 10-min intervals.

RESULTS

The results with the enzyme inducers are shown in Table 1. There was a significant increase of 33 per cent in liver blood flow and 26 per cent in liver weight with phenobarbital. Consequently blood flow/g of liver was unchanged. The increase in liver blood flow was entirely due to an increase in portal venous flow with hepatic arterial flow remaining constant (Fig. 1). The flow to the spleen did not change with phenobarbital and so the increase in portal flow was due to an increased flow to the gastrointestinal tract and pancreas (which were not separately assessed). Neither the spleen weight nor the combined weight of the pancreas and intestines was affected by any drug.

The other two enzyme inducers used, 3,4-benzpyrene and 3-methyl-cholanthrene, caused no hemodynamic changes but did cause a small but significant increase in liver weight relative to body weight (Table 1). Blood flow/g of liver was not significantly changed.

Enzyme induction occurred with all inducers, as judged by a decrease in zoxazolamine paralysis time. The animals induced with phenobarbital were paralyzed about 40 per cent of the time of the control animals (57.1 ± 8.3 min, phenobarbital group vs 137.1 ± 13.9 min, control group), whereas rats pretreated with 3,4-benzpyrene and 3-methylcholanthrene were not paralyzed at all with 60 mg/kg, bw of zoxazolamine.

The effects of SKF-525A are shown in Table 2. There were no significant changes in total liver blood flow. The rats treated with SKF-525A, however, did have a slightly larger portal flow (6.7 ± 0.3 ml/100 g, bw) and lower hepatic arterial flow (0.7 ± 0.07 ml/100 g, bw) than the comparable control group (5.6 ± 0.2 ml/100 g, bw, and 1.2 ± 0.2 mg/100 g, bw, respectively). With total liver blood flow being the same, these small differences are unlikely to be biologically significant. There were no other differences with SKF-525A.

DISCUSSION

This study clearly shows that phenobarbital is capable of increasing liver blood flow in the rat propor-

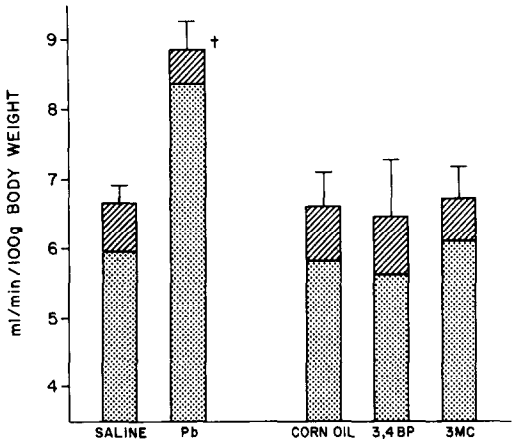

Fig. 1. Effects of enzyme inducers on liver blood flow, ml/min/100 g of body weight. Pb indicates phenobarbital-treated animals, 3,4-BP are 3,4-benzpyrene-treated, and 3-MC are 3-methylcholanthrene-treated animals. Hepatic arterial flow is represented by cross-hatched bars and splanchnic flow draining into the portal vein is represented by the dotted bars. The dagger(†) indicates measurements significant at level $P < 0.01$.

Table 2. Effects of SKF-525A on arterial pressure, cardiac output, liver weight and liver blood flow*

	Saline	SKF-525A
Body wt (g)	291.5 ± 6.5	294.5 ± 7.1
Mean arterial pressure (mm Hg)	129.4 ± 4.1	137.2 ± 3.2
Cardiac output (ml/min/100 g, bw)	26.8 ± 1.2	29.2 ± 2.1
Liver weight (g)	11.2 ± 0.7	11.2 ± 0.6
Liver weight/body weight × 100	3.82 ± 0.17	3.81 ± 0.17
Liver blood flow (ml/min/100 g, bw)	6.77 ± 0.26	7.47 ± 0.36
Liver blood flow (ml/min/g liver)	1.82 ± 0.12	1.98 ± 0.11

* Means ± standard errors of groups of thirteen rats.

tionately to the increase in liver mass. These data are similar to those recently reported by Ohnhaus and Locher [2] using colloidal gold clearance, but smaller than Ohnhaus *et al.* [1] originally found by a heat exchange method. Although Denis *et al.* [3] have reported no change in liver blood flow with phenobarbital, their mean data show an increase of 27 per cent in liver blood flow (from 6.64 to 8.43 ml/min/100 g, bw) similar to ours, but because of the scatter in the data, the results did not reach the level of significance. Our results in the rat are also consistent with those reported in the rhesus monkey [4], where equivalent increases in liver weight and liver blood flow were found.

The change in liver blood flow with phenobarbital was due entirely to an increase in splanchnic blood flow and in particular gastro-intestinal blood flow. Identical findings were found in the rhesus monkey treated with phenobarbital [4]. In neither instance was there an increase in intestinal weight. Although Ohnhaus [9] attempted to measure changes in distribution of blood flow to intestinal organs in the rat with ^{86}Rb, methodologic problems did not allow him to draw any conclusions regarding phenobarbital. The mechanism whereby this increased splanchnic blood flow occurs is unknown. It is known that small changes in portal venous pressure can influence mesenteric vascular resistance by myogenic or local reflex mechanisms whereby a rise in portal venous pressure raises mesenteric vascular resistance and vice versa [10]. Perhaps the increase in liver mass produced by phenobarbital results in a decrease in resistance to portal flow thereby triggering a decrease in mesenteric vascular resistance and an increase is splanchnic flow.

With the polycyclic hydrocarbons, however, we found no change in liver blood flow in spite of enzyme induction being achieved, as evidenced by reduced zoxazolamine paralysis time and a small but significant increase of 11–15 per cent liver weight relative to body weight. The liver blood flow/gram of liver was not significantly changed, although a less than 10 per cent change would probably not be detected by our method [7]. Ohnhaus *et al.* [1] studied a few animals receiving 3,4-benzpyrene by the heat exchange method and also found that it had no effect on liver blood flow. The differing effect on liver blood flow is only one of a number of important differences between polycyclic hydrocarbons and phenobarbital [11]. The lesser effect of the polycyclic hydrocarbons on liver weight is well known [12, 13], and there are marked differences in the ultrastructural changes in the liver produced by phenobarbital and the polycyclic hydrocarbons, a much greater increase in smooth endoplamsic reticulum being seen with phenobarbital treatment [14] Additionally, the spectrum of enzymes induced is more limited with the polycyclic hydrocarbons than with phenobarbital [11].

Inhibition of microsomal enzyme activity with SKF-525A did not change liver blood flow. Whereas this is not surprising, it is at variance with the conclusions drawn by Marchand and Brodeur [5] from studies using colloidal gold clearance to estimate hepatic blood flow. These authors showed that colloidal gold clearance is decreased by 40 per cent. Our results suggest that the colloidal gold clearance may have been altered by SKF-525A independent of any effect on hepatic blood flow, and implies an effect of SKF-525A on the reticuloendothelial system, a possibility which heretofore has been felt not to exist, but which is certainly worth further investigation.

Of the agents tested which affect hepatic enzyme activity, then, only induction of microsomal enzymes with phenobarbital produced changes in hepatic blood flow which could affect the metabolic clearance *in vivo* of drugs with a large hepatic extraction and whose clearance is therefore flow dependent [4]. This effect on blood flow may account for some of the observed differences *in vivo* resulting from induction with phenobarbital or polycyclic hydrocarbons.

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