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DIFFERENTIAL EFFECTS OF HEPATIC MICROSOMAL ENZYME INDUCING AGENTS ON LIVER BLOOD FLOW*

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Abstract—Male and female rats were injected i.p. for 5 days with phenobarbitone (80 mg/kg/day), antipyrine (80 mg/kg/day), phenytoin (65 mg/kg/day) or chlordiazepoxide (40 mg/kg/day). Seven days after start of treatment, radioactive microspheres were used to determine liver blood flow in some animals from treatment groups. Other animals were used to measure liver microsomal protein, cytochrome *c* reductase and cytochrome P450. Pentobarbitone sleeping time was also determined. In males, all the drugs significantly increased cytochrome P450 content and liver weight/100 g body weight (bw) relative to saline-treated controls. Also, pentobarbitone sleeping time was significantly decreased by all 4 drugs. However, only phenobarbitone changed liver blood flow: liver weight was increased by 23 per cent and this was paralleled by a 32 per cent increase in liver blood flow/100 g bw. Female rats receiving saline had lower liver cytochrome P450 contents and longer pentobarbitone sleeping times than the control males. In addition, the drugs were less potent in the females; all significantly reduced sleeping time but liver weight/100 g bw and cytochrome P450 content were only significantly increased by phenobarbitone and phenytoin. After phenytoin, antipyrine and chlordiazepoxide, liver blood flows/100 g bw were within 4 per cent of the control value whereas with phenobarbitone there was a 9 per cent increase which accompanied an 11 per cent increase in liver weight/100 g bw. The dose-effect relations of phenobarbitone were determined in male rats using doses of 5, 10 and 80 mg/kg/day and some animals were given amylobarbitone (80 mg/kg/day) in order to see if it also changed liver blood flow. Phenobarbitone gave dose-dependent effects on the biochemical parameters, liver weight/100 g bw and liver blood flow/100 g bw. Amylobarbitone was much less potent than phenobarbitone but did cause parallel changes in liver blood flow and liver weight/100 g bw. It is concluded that, of all the hepatic microsomal enzyme inducing agents which have been studied, only barbiturates increase both liver blood flow and liver weight.

Many drugs are known to change the efficacy of other therapeutic agents with which they are administered. In a number of cases the basis of this interaction is known to be the induction of hepatic microsomal drug metabolizing enzymes but, as in the case of phenobarbitone, the change in enzyme activity is not always sufficient to explain the changes in the rates of disappearance of other drugs from plasma. It is possible that such inducing agents also change the flow of blood through the liver. Consequently, the relationship between liver blood flow and hepatic microsomal enzyme activity has been investigated by a number of workers.

Enzyme inducing agents which have been studied in order to see if they change liver blood flow include phenobarbitone [1–5], antipyrine [1], 3,4-benzpyrene [1, 5] and 3-methylcholanthrene [5]. Of these drugs only phenobarbitone [1, 2, 4, 5] and antipyrine [1] have been reported to increase liver blood flow. The effects of enzyme inhibition with SKF-525A have also been investigated; Marchand and Brodeur [6] found that this agent decreased colloidal gold clearance in the rat and interpreted this as a decrease in blood flow whilst Nies *et al.* [5] found no change in liver blood flow as determined by radioactive microspheres.

In order to investigate this matter further we have used radioactive microspheres to determine liver blood flow in rats which have been treated with a number of therapeutic agents known to interact with other drugs. We have also investigated the effects of several doses of phenobarbitone in order to see if the effects it produces are dose-dependent.

METHODS

Enzyme induction. Wistar rats weighing 250–350 g were given intraperitoneal (i.p.) injections of the microsomal enzyme inducing agents once (chlordiazepoxide) or twice (all other agents) daily for 5 days. Determinations of liver blood flow or microsomal enzyme activity were carried out on the 7th day. All drugs, except phenytoin, were injected in a volume of 100 μl physiological saline/100 g body weight (bw). Phenytoin was given in 200 μl physiological saline containing 0.01 M sodium hydroxide/100 g bw. Two control groups were used: one group was injected i.p. with 100 μl physiological saline/100 g bw and the other with 200 μl 0.01 M sodium hydroxide in physiological saline/100 g bw. Rats in treatment groups were randomly allocated to blood flow or enzyme studies.

Measurement of liver blood flow. Animals were

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anaesthetized with sodium pentobarbitone (60 mg/kg i.p.; Sagatal, May & Baker). Arterial blood pressure was measured from one femoral artery whilst the other femoral artery was cannulated to allow withdrawal of blood at a constant rate (0.6 ml/min) during and for 70 sec after injection of the microspheres. Carbonised microspheres of $15 \pm 5\,\mu m$ diameter, labelled with ^{85}Sr (3M Co., St Paul, Minnesota), were injected into the left ventricle through a cannula passed down the right carotid artery. The microspheres were injected over 20 sec in 0.6 ml of 0.2% Tween 80 in physiological saline. Cardiac output and liver blood flow were calculated as described by Nies *et al.* [5]. In this method, hepatic arterial flow is determined from the microspheres trapped in the liver and portal venous return is obtained indirectly by adding together the flows to the spleen, pancreas and gastrointestinal tract. Throughout this paper, liver blood flow refers to the sum of hepatic arterial and portal venous flows.

In vitro assays. Rats were killed by cervical dislocation, the livers rapidly removed and homogenised in ice-cold 1.15% KCl using a Teflon in glass homogeniser. The 30% homogenate was centrifuged at 10,000 *g* for 20 min at 4°. The resulting supernatant was decanted without disturbing the pellet and centrifuged at 105,000 *g* for 60 min at 4°. The microsomal pellet was resuspended in 10 ml of 0.2 ml phosphate buffer, pH 7.4. Microsomal protein, cytochrome P450 and cytochrome *c* reductase were determined respectively by the methods of Lowry *et al.* [7], Omura and Sato [8] and Williams and Kamin [9].

Pentobarbitone sleeping time. Groups of 4 Wistar rats were injected with inducing agents as described above. On the 7th day after start of the treatment, the duration of pentobarbitone (40 mg/kg i.p.) induced sleep was determined. Sleeping time was defined as the time between loss and return of the righting reflex.

RESULTS

There were no significant differences in liver weight, liver blood flow, microsomal protein or enzyme activity between rats receiving 0.1 ml physiological saline/100 g bw and those receiving 0.2 ml 0.01 M sodium hydroxide in physiological saline/100 g bw. The control groups in Tables 1–3 are those which received physiological saline.

Table 1 shows the effects of several inducing agents in male rats. Phenobarbitone (80 mg/kg/day), chlordiazepoxide (40 mg/kg/day), antipyrine (80 mg/kg/day) and phenytoin (65 mg/kg/day) all produced significant increases in liver weight/100 g bw. Phenobarbitone produced the greatest increase (23 per cent) and chlorodiazepoxide the smallest (10 per cent) with antipyrine (15 per cent) and phenytoin (18 per cent) having intermediate effects. All the drugs also significantly increased cytochrome P450 content and pentobarbitone sleeping time. Again, the phenobarbitone treatment produced the greatest changes; cytochrome P450 content was increased by 117 per cent relative to the control group whilst it increased by 36, 42 and 51 per cent respectively for chlordiazepoxide, antipyrine and phenytoin. The order of effectiveness on sleeping time was slightly different with

Table 1. Effects of hepatic microsomal enzyme inducing agents on liver weight, liver enzyme activity and liver blood flow in male rats*

	Saline	Chlordiazepoxide (40 mg/kg/day)	Antipyrine (80 mg/kg/day)	Phenytoin (65 mg/kg/day)	Phenobarbitone (80 mg/kg/day)
Body weight (g)	287 ± 16 (14)	297 ± 20 (11)	315 ± 16 (12)	281 ± 19 (12)	307 ± 18 (14)
Cardiac output (ml/min/100g bw)	22.2 ± 3.2 (7)	20.8 ± 1.0 (7)	22.7 ± 1.6 (7)	20.7 ± 0.8 (7)	23.9 ± 0.7 (6)
Mean arterial pressure (mm Hg)	116 ± 7 (7)	125 ± 5 (7)	126 ± 5 (7)	122 ± 4 (7)	124 ± 7 (6)
Liver weight (g/100 g bw)	3.83 ± 0.11 (14)	4.23 ± 0.10 (11)‡	4.41 ± 0.15 (12)‡	4.52 ± 0.17 (11)§	4.79 ± 0.14 (14)§
Liver blood flow (ml/min g liver)	1.33 ± 0.15 (7)	1.19 ± 0.05 (7)	1.17 ± 0.12 (7)	1.12 ± 0.09 (7)	1.36 ± 0.14 (6)
Liver blood flow (ml/min/100g bw)	5.20 ± 0.23 (7)	5.00 ± 0.29 (7)	4.74 ± 0.43 (7)	4.81 ± 0.30 (7)	6.87 ± 0.32 (6)‡
Microsomal protein (mg g liver)	16.4 ± 0.8 (6)	16.1 ± 0.9 (4)	15.5 ± 0.8 (4)	18.8 ± 4.2 (4)	20.4 ± 1.7 (4)†
Cytochrome P450 (nmol mg protein)	0.93 ± 0.09 (6)	1.27 ± 0.12 (4)†	1.32 ± 0.12 (4)†	1.40 ± 0.23 (4)†	2.02 ± 0.14 (4)§
Cytochrome *c* reductase (nmol/mg protein/min)	47.5 ± 4.4 (6)	59.6 ± 6.4 (4)	58.7 ± 6.3 (4)	78.9 ± 10.4 (4)†	61.1 ± 6.1 (4)†
Sleeping time (min)	104 ± 13.5 (4)	36.1 ± 3.0 (4)§	56.2 ± 14.3 (4)†	32.5 ± 2.5 (4)§	4.7 ± 3.0 (4)§

* Values are given as mean ± S.E.M. with the number of animals in parentheses.
Student's un-paired *t*-test was used in order to determine statistical significance with respect to the saline treated animals. † $P < 0.05$, ‡ $P < 0.001 \leq P < 0.001$.

Table 2. Effects of hepatic microsomal enzyme inducing agents on liver weight, liver enzyme activity and liver blood flow in female rats*.

	Saline	Chlordiazepoxide (40 mg/kg/day)	Antipyrine (80 mg/kg/day)	Phenytoin (65 mg/kg/day)	Phenobarbitone (80 mg/kg/day)
Body weight (g)	254 ± 19 (11)	251 ± 18 (10)	258 ± 17 (11)	243 ± 7 (10)	259 ± 8 (13)
Cardiac output (ml/min/100 g bw)	21.2 ± 2.1 (7)	20.4 ± 1.7 (6)	23.4 ± 1.6 (6)	21.2 ± 2.1 (6)	20.7 ± 2.8 (6)
Mean arterial pressure (mm Hg)	118 ± 6 (7)	121 ± 4 (6)	123 ± 3 (6)	125 ± 3 (6)	119 ± 5 (6)
Liver weight (g/100g bw)	3.45 ± 0.13 (11)	3.69 ± 0.17 (10)	3.71 ± 0.18 (11)	4.43 ± 0.05 (10)§	3.84 ± 0.18 (13)†
Liver blood flow (ml/min/g liver)	1.40 ± 0.06 (7)	1.42 ± 0.17 (6)	1.42 ± 0.24 (6)	1.15 ± 0.21 (6)	1.45 ± 0.31 (6)
Liver blood flow (ml/min/100g bw)	4.97 ± 0.29 (7)	4.80 ± 0.46 (6)	4.92 ± 0.56 (6)	5.03 ± 0.90 (6)	5.41 ± 0.62 (6)
Microsomal protein (mg/g liver)	13.9 ± 1.3 (4)	15.3 ± 1.0 (4)	13.8 ± 0.6 (4)	13.0 ± 1.4 (4)	14.3 ± 1.8 (4)
Cytochrome P450 (nmol/mg protein)	0.73 ± 0.03 (4)	0.80 ± 0.06 (4)	0.65 ± 0.22 (4)	0.91 ± 0.07 (4)†	0.98 ± 0.08 (4)†
Cytochrome *c* reductase (nmol/mg protein/min)	47.0 ± 3.1 (4)	50.9 ± 2.2 (4)	41.3 ± 5.0 (4)	61.2 ± 5.2 (4)†	60.5 ± 7.2 (4)†
Sleeping time (min)	242 ± 16.0 (4)	98.6 ± 13.3 (4)§	179 ± 13.0 (4)†	49.1 ± 7.2 (4)§	31.1 ± 8.2 (4)§

* Values are given as mean ±S.E.M. with the number of animals in parentheses.
Student's un-paired *t*-test was used in order to determine statistical significance with respect to the saline treated animals. † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$.

Table 3. Effects of phenobarbitone and amylobarbitone on liver weight, liver enzyme activity and liver blood flow in male rats*.

	Saline	Amylobarbitone (80 mg/kg/day)	Phenobarbitaone		
			5 mg/kg day	10 mg/kg/day	80 mg kg day
Body weight (g)	287 ± 16 (14)	317 ± 8 (10)	312 ± 13 (10)	323 ± 13 (10)	307 ± 18 (14)
Cardiac output (ml/min/100 g bw)	22.2 ± 3.2 (7)	21.9 ± 0.3 (6)	22.3 ± 0.3 (6)	22.3 ± 0.5 (6)	23.9 ± 0.7 (6)
Mean arterial pressure (mm Hg)	116 ± 7 (7)	121 ± 5 (6)	124 ± 1 (6)	123 ± 2 (6)	124 ± 7 (6)
Liver weight (g/100 g bw)	3.83 ± 0.11(14)	4.18 ± 0.09 (10)†	4.19 ± 0.16 (10)†	4.41 ± 0.08 (10)§	4.70 ± 0.14(14)§
Liver blood flow (ml/min/g liver)	1.33 ± 0.15 (7)	1.37 ± 0.07 (6)	1.40 ± 0.06 (6)	1.39 ± 0.06 (6)	1.36 ± 0.14 (6)
Liver blood flow (ml/min/100 g bw)	5.20 ± 0.23 (7)	5.80 ± 0.41 (6)	5.84 ± 0.02 (6)†	6.12 ± 0.22 (6)†	6.87 ± 0.32 (6)‡
Microsomal protein (mg/g liver)	16.4 ± 0.8 (6)	17.4 ± 0.5 (4)	16.5 ± 1.0 (4)	18.7 ± 0.9 (4)†	20.4 ± 1.7 (4)†
Cytochrome P450 (nmol/mg protein)	0.93 ± 0.09 (6)	1.10 ± 0.07 (4)	1.37 ± 0.13 (4)‡	1.33 ± 0.04 (4)§	2.02 ± 0.14 (4)§
Cytochrome *c* reductase (nmol/mg protein/min)	47.5 ± 4.4 (6)	50.9 ± 4.7 (4)	48.0 ± 3.5 (4)	59.1 ± 5.7 (4)	61.1 ± 6.1 (4)†
Sleeping time (min)	104 ± 13.5 (4)	58.0 ± 5.0 (4)‡	43.2 ± 3.6 (4)§	29.3 ± 1.8 (4)§	4.7 ± 3.0 (4)§

* Values are given as mean ±S.E.M. with the number of animals in parentheses.
Student's un-paired *t*-test was used in order to determine statistical significance with respect to the saline treated animals. † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$.

chlordiazepoxide giving a shorter period of immobilization than antipyrine.

In contrast, only the phenobarbitone treatment significantly changed liver blood flow/100 g bw. The percentage increase (32 per cent) was similar to the increase in liver weight/100 g bw with the result that liver blood flow/g liver remained virtually the same as controls. The other three treatments resulted in falls in liver blood flow/g liver and liver blood flow/100 g bw. Here, chlordiazepoxide, which produced the least increase in liver weight, gave a liver blood flow closest to the control value whilst phenytoin treatment, which gave the greatest change in liver weight apart from phenobarbitone, resulted in the lowest rate of liver perfusion.

Table 2 shows that the drugs were not as effective in female rats as in male animals. All of them significantly shortened pentobarbitone sleeping time but only phenobarbitone and phenytoin produced significant increases in any of the other indices of induction. Following administration of these two drugs, liver weight/100 g bw, cytochrome P450 content and cytochrome *c* reductase activity were significantly greater than in the control group of animals. However, in the female rats there were no significant changes in blood flow although phenobarbitone treatment produced an increase of 9 per cent in liver blood flow/100 g bw. Phenytoin produced the largest increase in liver weight (35 per cent) in the female rats and it is only with this drug that there is a fall in liver blood flow/g liver to a rate similar to those found with chlordiazepoxide, antipyrine and phenytoin in the male rats.

Table 3 gives the results of experiments performed in order to see if the effects of phenobarbitone were dose-dependent and if another barbiturate shared its property of increasing liver blood flow. Phenobarbitone produced dose-dependent increases in both liver weight/100 g bw and liver blood flow/100 g bw at doses of 5, 10 and 80 mg/kg/day. The increases in liver weight/100 g bw were 9, 15 and 22 per cent respectively and the changes in liver blood flow/100 g bw were 12, 18 and 32 per cent. Amylobarbitone (80 mg/kg/day) produced a lesser degree of induction than the lowest dose of phenobarbitone but it did give an 11 per cent increase in liver blood flow/100 g bw which paralleled the 8.7 per cent increase in liver weight/100 g bw.

All the barbiturate-induced increases in liver blood flow were the result of increased return of blood from the gastrointestinal tract and pancreas rather than increased flow through the hepatic artery.

DISCUSSION

The present study considerably increases the number of drugs and variety of chemical structures which have been investigated in order to see if they produce changes in liver blood flow consequent upon their effects on hepatic microsomal drug metabolizing enzymes. Our results with phenobarbitone confirm the findings of Ohnhaus *et al.* [1, 2] and Nies *et al.* [5] who demonstrated that an increase in liver blood flow accompanied the increase in liver weight produced by treatment of rats with this drug. A similar increase in liver blood flow occurs after administration of phenobarbitone to rhesus monkeys [4]. The results of Denis *et al.* [3] also show an increase in liver blood flow after phenobarbitone treatment of rats, but is was not significant.

Of the other agents that have previously been studied only antipyrine has been reported to increase liver blood flow [1]. However, the heat exchange method used by Ohnhaus *et al.* is only semiquantitative and the changes in liver heat conductivity ranged from 33 to 175 per cent for phenobarbitone which is very much larger than the changes in liver blood flow determined with microspheres or colloidal gold. Nies *et al.* [5] found blood flow increased by 33 per cent in their study using microspheres, our study shows phenobarbitone (80 mg/kg/day) produced a 32 per cent increase and Ohnhaus and Locher [2] demonstrated a 27 per cent increase with colloidal gold. Since these last three studies used daily doses of between 30 and 80 mg/kg/day and treatment was 3, 5 or 10 days, the very much greater increase implied by the heat exchange method cannot be explained by larger doses or longer treatment since the animals in that study were treated with 30 mg/kg/day for 4 days. These discrepancies between the heat exchange method and the more direct measurements of blood flow suggest that the former may be affected by other factors as well as hepatic blood flow and this may explain why our results with antipyrine are at variance with those of Ohnhaus *et al.* [1].

Since phenobarbitone is the most potent of the commonly used experimental hepatic microsomal enzyme inducing agents we studied the effects of several doses in order to see if the increase in liver blood flow only occurred after a threshold degree of induction had been exceeded. Doses as small as 5 mg/kg/day gave significant increases in liver weight/100 g bw and liver blood flow/100 g bw. The increase in liver weight/100 g bw was less than that given by treatment with antipyrine, phenytoin or chlordiazepoxide but the low dose of phenobarbitone still gave a significant increase in liver blood flow/100 g bw. Thus the increase in liver blood flow is not a phenomenon which occurs after induction has caused a certain increase in liver size.

The rats treated with amylobarbitone showed greater variation in liver blood flow/100 g bw than did those treated with phenobarbitone. Consequently the increase over the control value did not reach statistical significance. The treatment used did not produce the same degree of induction as the lowest dose of phenobarbitone as determined by cytochrome P450 content or pentobarbitone sleeping time. However, on the basis of increase in liver weight the amylobarbitone produced a similar degree of induction as the chlordiazapoxide treatment whilst on the basis of pentobarbitone sleeping time it is approximately equipotent with antipyrine. Both these non-barbiturate drugs decreased liver blood flow and thus it would seem that amylobarbitone, alone of the drugs that we have studied, shares with phenobarbitone the property of increasing liver blood flow in parallel with increasing liver weight.

Our discussion so far has been limited to experiments in which male animals were used and in our study we found that female rats were considerably different from the male rats investigated. For example, the cytochrome P450 content of the livers of the control

female rats was only 78 per cent of that found in the control males and their pentobarbitone sleeping time was 2.3 times as long. Microsomal protein content was also significantly lower in the female rat livers. Not only did untreated female rats appear to have lower drug metabolizing activity than comparative males, but those treated with hepatic microsomal enzyme inducing agents responded less than treated males. Significant increases in cytochrome P450 content only occurred after treatment with phenobarbitone (80 mg/kg/day) and phenytoin, whereas all four drugs gave statistically significant increases in male rats. Also, liver weight/100 g bw increased by 7.0, 7.5, 11 and 35 per cent respectively in females treated with chlordiazepoxide, antipyrine, phenobarbitone and phenytoin compared with increases in the male rats of 10, 15, 23 and 18 per cent. The greater increase with phenytoin in the females may be related to their being the only group which lost weight during treatment.

Examination of the values obtained for liver blood flow/100 g bw reveals that, after chlordiazepoxide, antipyrine and phenytoin, they were between 96.5 and 101 per cent of the control values. On the other hand, the phenobarbitone treatment resulted in a statistically non-significant 9 per cent increase in liver blood flow/100 g bw which accompanied the 11 per cent increase in liver weight/100 g bw. Thus 80 mg/kg/day phenobarbitone given to females not only produced a similar increase in liver weight/100 g bw to that given by 5 mg/kg/day in males but also resulted in a similar increase in liver blood flow/100 g bw. Therefore, it would seem that phenobarbitone increases liver blood flow in proportion to the increase it produces in liver weight in both male and female animals.

In both male and female rats the increases in liver blood flow after administration of phenobarbitone and amylobarbitone were entirely due to increases in portal venous return. Nies *et al.* [5] suggested that, as a result of the increase in liver size, phenobarbitone might decrease hepatic resistance to portal venous flow and thus lower portal venous pressure. They pointed out that portal venous pressure is known to affect vascular resistance [10] and suggested that if phenobarbitone decreases portal venous pressure, a fall in mesenteric vascular resistance may follow and thus produce an increase in blood flow through the splanchnic bed. Whatever the mechanism of the increase in blood flow with the barbiturate drugs examined, our results and those of Nies *et al.* [5] show that an increase in liver size as a result of treatment with an enzyme inducing agent does not itself produce an increase in liver blood flow. It is likely, therefore, that the increases in liver mass produced by the barbiturates are qualitatively different from those given by the other hepatic microsomal enzyme inducing agents that have been studied.

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