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Effects of phenobarbitone and 6-methylprednisolone pretreatment on pressure/flow relations in the superior mesenteric and iliac arterial beds of the rat

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The in-situ blood perfused rat superior mesenteric arterial and right iliac arterial preparations were used to investigate the effects of pretreatment for 5 days with either phenobarbitone (80 mg kg^{-1} daily) or 6-methylprednisolone (17 mg kg^{-1} daily) on regional vascular resistances. The elevation of the regression of perfusion pressure on perfusion rate in the superior mesenteric arterial bed was significantly decreased by both phenobarbitone and 6-methylprednisolone over the range of flow rates used. Neither phenobarbitone nor 6-methylprednisolone had a significant effect on hepatic portal venous pressure nor was there any significant effect on the regression of perfusion pressure on flow rate in the in-situ iliac arterial bed. It is concluded that pretreatment with either phenobarbitone or 6-methylprednisolone lowers vascular resistance in the superior mesenteric arterial by approximately 9 and 8% respectively but does not affect vascular resistance in the iliac arterial bed.

The hepatosplanchnic circulation receives approximately one-fifth of the cardiac output and in recent years certain therapeutic agents have been shown to increase blood flow in the hepatosplanchnic bed by bringing about a redistribution of the cardiac output in favour of the organs draining into the hepatic portal vein. Thus, pretreatment of rats with phenobarbitone (Nies et al 1976; Yates et al 1978), 6-methylprednisolone (Wilson & Hiley 1983) or ICI 53072 (Berman et al 1983) increases hepatosplanchnic blood flow. However the mechanisms by which these increases in liver blood flow are brought about are unclear. Hepatomegaly and enzyme induction do not always increase portal venous return (Yates et al 1978). Also, the means by which these drugs affect liver size, that is by hypertrophy or by hyperplasia, is not correlated with the ability to enhance its blood supply (Berman et al 1983; Wilson & Hiley 1983); indeed, 6-methylprednisolone does not cause an increase in absolute liver size (Wilson & Hiley 1983). Therefore, it does not appear that there is any common structural change in the liver produced by these drugs which could account for the increase in hepatosplanchnic blood flow.

The liver is unusual in that it receives a dual blood supply from the hepatic artery and the portal vein. The hepatic artery accounts for between 5 and 20% of the total liver blood flow and thus the greater proportion of the hepatic blood supply is derived from the portal vein, the recipient of the drainage from the intestine, pancreas and spleen. Thus, under normal conditions, virtually all the blood entering the splanchnic circulation through the coeliac, superior mesenteric and inferior mesenteric arteries drains into the portal vein and, in the rat, the organs supplied by the superior mesenteric artery provide about 75% of portal venous flow (Wilson 1981). Portal venous pressure in this species is approximately 8 mmHg (Vorobioff et al 1983) whereas that in the superior mesenteric artery (mean arterial pressure) is 100–120 mmHg and hepatic venous pressure is about 3 mmHg. Thus the mesenteric arteriolar bed is the major source of resistance to flow from the superior mesenteric artery to the hepatic vein and any change in resistance here may have a profound effect on portal venous flow and, hence, total hepatic blood flow. Accordingly, we have investigated the effects of pretreatment with 6-methylprednisolone and phenobarbitone on pressure/flow relations in the blood perfused superior mesenteric artery preparation of the rat (Jackson & Campbell 1980). The blood perfused right iliac arterial bed was also studied in animals pretreated with the two drugs in order to determine whether or not any changes observed in the mesenteric arterial bed were to be found in other areas of the vasculature.

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METHODS

Animals and pretreatment

Groups of male Wistar rats (Tucks Ltd, Battlebridge, Essex), 200–260 g were fed on a standard laboratory diet (Labsure; C. Hill Ltd, Poole, Dorset) and kept in mesh-floor cages on a 12 h light/dark cycle. Weight-matched groups from a given batch were pretreated for 5 days with intraperitoneal (i.p.) injections of 0·9% NaCl (saline) (2 ml kg^{-1} twice daily) or phenobarbitone (40 mg kg^{-1} twice daily in saline) or 6-methylprednisolone (8·5 mg kg^{-1} of the parent steroid in saline twice daily: the preparation used was Solu-Medrone for injection, Upjohn). The animals were starved overnight before use in perfusion studies on the 6th day after commencement of pretreatment; this was considered a necessary precaution to minimize variations in liver blood flow consequent upon ingestion of food shortly before experimentation.

Pressure/flow studies on the superior mesenteric arterial bed

Pretreated rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.; Sagatal, May & Baker) and a tracheal cannula inserted to allow them to breathe spontaneously. A lateral tail vein was cannulated for the administration of additional anaesthetic and the left external jugular vein was cannulated to allow for the infusion of saline at a rate of 6 ml h^{-1} during the experiment and for bolus injections of heparin. The right common carotid artery was cannulated in order to record central arterial pressure by means of a Bell & Howell type 4-422-0001 pressure transducer connected to a Grass model 7D or 79D polygraph. Heart rate was derived from the pressure wave by means of a Grass tachograph preamplifier. Rectal temperature was maintained at 37 °C by means of a BioScience homeothermic blanket system.

The preparation used for the in-situ blood perfusion of the superior mesenteric arterial bed was similar to that described by Jackson & Campbell (1980). A mid-line incision was made in the abdomen and the intestines were displaced to allow positioning of cotton ligatures around the aorta distal to the origin of the renal arteries and around the superior mesenteric artery shortly after its origin. Surgical manipulation was stopped for 20 min to allow haemostasis before administration of 1000 units kg^{-1} heparin. The abdominal aorta was cannulated with PP60 tubing which led to a Harvard type 2903 servo-pump, a bubble trap and a heat exchanger for rewarming the blood to 37 °C. The circuit was filled with the animal's blood which was returned to the superior mesenteric artery at an initial rate of 2 ml min^{-1} by means of a PP50 cannula. During the cannulation procedure the superior mesenteric vascular bed was subjected to a period of ischaemia of less than 2 min. Input pressure was measured by means of a T-tube placed a fixed distance from the end of the cannula and a second Bell & Howell transducer. The preparation was allowed to stabilize for 20 min before commencement of the determination of the pressure/flow relationship using either seven or eight flow rates over a range of 0·4 to 3·04 or 3·54 ml min^{-1}. Flow rate was changed every 2 min according to a Latin Square design and each rate was used at least 4 times.

The pressure recorded is the sum of the vascular resistance and the resistance of the cannula through which the blood passes between the T-tube and the artery. Correction was made to the recorded pressure for the pressure drop in the cannula by means of recording the actual pressure in the cannula alone at each flow rate using the animal's blood at the end of the experiment and subtracting these values from the recorded pressures. This was found to give values within 5% of the actual pressures recorded when recordings were made from a branch of the superior mesenteric artery. Determinations were not routinely made in this way since they necessitate the tying off of a considerable part of the vascular bed. Arterial blood samples (120 µl) were taken from the carotid artery at the start and finish of the perfusion for determination of the blood pH, pO_2 and pCO_2 using a Corning 166 micro blood gas analyser. At the end of the experiment the liver was removed, blotted dry and weighed.

Pressure/flow relation in the perfused right iliac arterial bed

The perfusion circuit was the same as that employed for the mesenteric arterial bed and the surgical procedure followed a similar pattern. Blood was withdrawn from the left common carotid artery and returned into the right iliac artery just after the bifurcation of the abdominal aorta. Central arterial pressure was monitored from the left femoral artery. Again the perfused vascular bed experienced only a brief period of ischaemia and a period of 20 min was allowed to elapse after commencement of the perfusion with a flow rate of 2 ml min^{-1}. Eight flow rates ranging from 0·4 to 3·54 ml min^{-1} were used to determine the pressure/flow relations of this vascular bed. Blood gases were determined as before and animals showing severe deterioration were excluded

from the study. Liver weight was determined at the end of each experiment.

Determination of hepatic portal venous pressure

This was measured by the insertion of a 21 g stainless steel needle cannula into the portal vein proper. The needle was connected to a Bell & Howell pressure transducer by PP60 tubing such that the total length of needle and tubing was 6 cm; the tubing and transducer were filled with 0·9% NaCl. The transducer was connected to the Grass 7D polygraph and was calibrated daily for venous pressures with a water manometer (0–25 cm H_2O). The pressure reading was taken when it was stable and clear breathing variations were observed.

Statistical methods

All values are given as the mean ± 1 s.e.m. and the number of animals in a group is represented by n. Comparison between an experimental mean and the corresponding control value was carried out by Student's *t*-test. Comparison of the regression of perfusion pressure on flow rate was made by means of analysis of covariance with correction being made for the curvature of the lines (Snedecor & Cochran 1980). $\bar{y}$ Refers to the mean elevation of the regression line at the point $(\bar{x}, \bar{y})$; values for $\bar{x}$ are not given since they were identical for each investigation as the same flow rates were used for both the control and experimental animals in a given comparison.

RESULTS

Perfusion of the superior mesenteric arterial bed

Liver weight in the saline-pretreated group of rats was 3·05 ± 0·06 g/100 g body weight (n = 10) and in the corresponding phenobarbitone pretreated group it was 3·90 ± 0·06 g/100 g body weight (n = 9); an increase of 27·9% ($P < 0·001$; Students *t*-test). Mean arterial pressure was 122 ± 3 and 120 ± 6 mmHg in the saline and phenobarbitone groups respectively. Fig. 1a shows the relation of superior mesenteric perfusion pressure to flow rate in these two groups of rats. The mean pressure at all of the eight flow rates used was significantly lower in the phenobarbitone-pretreated group. Analysis of covariance showed that, although the slopes were not different (8·76 ± 0·24 and 8·27 ± 0·18 mmHg min ml^{-1} for control and phenobarbitone respectively), the elevation of the regression line of perfusion pressure on flow rate at the mean point $(\bar{x}, \bar{y})$ was significantly lower ($P <$ 0·001) in the phenobarbitone pretreated rats, where $\bar{y}$ had a value of 25·3 ± 1·0 mmHg, than in the saline pretreated group where $\bar{y}$ was 27·8 ± 1·0 mmHg.

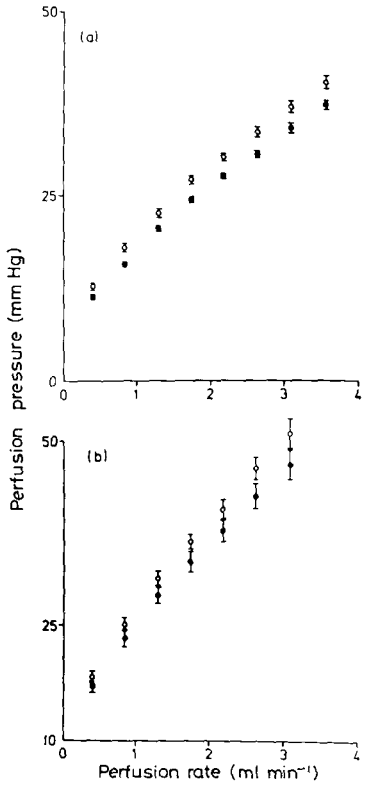

FIG. 1. Pressure/flow relations in the blood perfused superior mesenteric artery preparation. (a) Effects of pretreatment of rats with saline (4 ml kg^{-1} daily for 5 days) or phenobarbitone (80 mg kg^{-1} daily for 5 days) given i.p. (○) control rats pretreated with saline (n = 10) and (●) animals given phenobarbitone (n = 9). (b) Effects of pretreatment of rats with saline or 6-methylprednisolone (17 mg kg^{-1} daily for 5 days). (○) saline pretreated rats (n = 10) and (●) rats pretreated with the glucocorticoid. The points represent the mean with the bars representing ± 1 s.e.m. for the number of rats in the group.

Pretreatment of rats with 6-methylprednisolone significantly increased ($P < 0·01$) the liver weight to body weight ratio from 3·13 ± 0·10 to 3·50 ± 0·11 g/100 g body weight (n = 10 for both groups). However, this was the result of a significantly lower rate of weight gain in the steroid-treated animals; the control animals had increased body weight by 5·6 ± 0·8% during the treatment period whilst the treated animals lost 2·6 ± 0·7% of their initial body weight ($P < 0·001$). Mean arterial pressure was not significantly different in the two groups being 121 ± 3 mmHg in the control animals and 118 ± 5 mmHg in the steroid treated rats. Fig. 1b illustrates the pressure/flow relations in these two groups of animals and it is notable that with 6-methylprednisolone pretreatment, as with phenobarbitone pretreatment,

mean perfusion pressure at each flow rate was lower than in the saline-pretreated controls. In this case also, the mean elevation of the lines was significantly different ($P < 0{\cdot}01$; analysis of covariance) with $\bar{y}$ for the control being 35·5 ± 1·4 mmHg whilst that for the steroid pretreated group was 32·8 ± 1·3 mmHg. The slopes of the regression lines through the mean point were not significantly different; that for the controls was 12·1 ± 0·5 mmHg min ml^{-1} and that for the experimental group was 11·0 ± 0·6 mmHg min ml^{-1}.

Effects of phenobarbitone and 6-methylprednisolone on hepatic portal venous pressure

Portal venous pressure was not significantly changed by pretreatment with either drug when the mean values were compared with the appropriate saline control group by Student's *t*-test. In the phenobarbitone group mean portal pressure was 6·5 ± 0·7 mmHg (n = 8) compared with a control value of 7·9 ± 1·0 mmHg (n = 9). Mean hepatic portal venous pressure following pretreatment with 6-methylprednisolone was 9·0 ± 0·7 mmHg (n = 7) and that in the corresponding control group was 7·8 ± 0·8 mmHg (n = 10).

Perfusion of the iliac arterial bed

Fig 2a shows the results obtained from a comparison of saline and phenobarbitone pretreatments on pressure/flow relations in this vascular bed. In these experiments the mean values for the perfusion pressures at each flow rate for the barbiturate-treated group were greater than those in the control rats, but not significantly so. Analysis of covariance showed that neither the slopes of the regression at the mean point (15·7 ± 1·1 mmHg min ml^{-1}, control; 16·5 ± 1·2 mmHg min ml^{-1}, phenobarbitone) nor the elevation of the lines ($\bar{y}$ = 51·0 ± 2·1 mmHg, control; $\bar{y}$ = 53·8 ± 2·2 mmHg, phenobarbitone) were significantly different between the groups. The mean liver weight to body weight ratio in the phenobarbitone group (4·20 ± 0·09 g/100 g body weight, n = 11) was 23·2% greater than in the control group (3·41 ± 0·08 g/100 g body weight, n = 10).

As observed previously, treatment of rats with 6-methylprednisolone significantly ($P < 0{\cdot}001$) reduced body weight gain relative to saline-pretreated animals. In this experiment the control rats increased mean body weight by 0·8 ± 1·0% (n = 10) whereas the experimental group lost 5·2 ± 0·7%

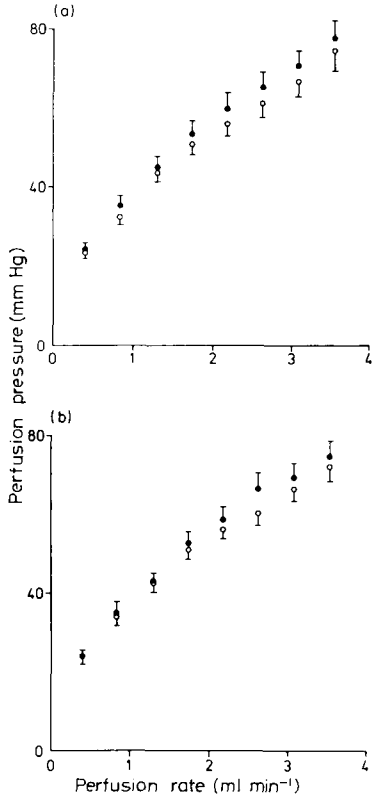

FIG. 2. Pressure/flow relations in the blood perfused iliac arterial bed. (a) Effects of pretreatment of rats with 0·9% NaCl solution (4 ml kg^{-1} daily for 5 days) or phenobarbitone (80 mg kg^{-1} daily for 5 days) given i.p. (○) Control rats pretreated with saline (n = 11) and (●) animals given phenobarbitone (n = 11). (b) Effects of pretreatment of rats with saline or 6-methylprednisolone (17 mg kg^{-1} daily for 5 days). (○) Saline-pretreated rats (n = 10) and (●) rats pretreated with the glucocorticoid (n = 9). The points represent the mean with the bars representing 1 s.e.m. for the number of rats in the group.

(n = 9). Mean liver weight to body weight ratio was unchanged, being 2·99 ± 0·09 g/100 g body weight (n = 10) in the saline group and 2·98 ± 0·06 g/100 g body weight (n = 9) in the steroid-pretreated animals. Fig. 2b shows that mean perfusion pressure at each flow rate was greater in the 6-methylprednisolone-pretreated group than in the control and the mean elevation of the regression line was not significantly different between the groups ($\bar{y}$ = 50·7 ± 2·0 mmHg, control; $\bar{y}$ = 52·9 ± 2·3 mmHg, 6-methylprednisolone). Also there was no difference between the slopes of the lines at the mean point (15·0 ± 0·9 and 16·1 ± 1·1 mmHg min ml^{-1} for the control and the 6-methylprednisolone groups respectively).

DISCUSSION

Previous investigations have shown that both phenobarbitone and 6-methylprednisolone, at the doses employed in this study, cause a redistribution of cardiac output in favour of the organs in the hepatosplanchnic bed (Yates et al 1978; Wilson & Hiley 1983). This is brought about almost entirely by increasing the blood flow to the organs draining into the hepatic portal vein and without increasing cardiac output itself (Nies et al 1976; Yates et al 1978; Wilson 1981; Wilson & Hiley 1983). We show here that pretreatment of rats with either drug did not cause a change in mean arterial pressure and thus the increase in hepatosplanchnic blood flow must be the result of a lower resistance in this bed relative to the other systemic vascular beds.

The superior mesenteric artery carries the largest proportion of the hepatosplanchnic blood flow and is, therefore, a likely site at which a decreased resistance might be detected should it be the basis of the change in relative vascular resistances. In this study we have shown that perfusion pressure in the superior mesenteric vascular bed, over the range of flow rates used, is reduced by pretreatment with either phenobarbitone or 6-methylprednisolone. Perfusion pressure was reduced fairly consistently by about 9·4 ± 0·6% (n = 8) and 7·6 ± 0·2% (n = 7) respectively as result of pretreatment with the barbiturate and the glucocorticoid and this suggests that resistance to flow is lower following the administration of these compounds. If portal venous pressure remains constant at flow rates between 0·4 and 3·5 ml min^{-1}, then these data suggest that vascular resistance has fallen by less than 10% although, previously, the proportion of cardiac output passing to tissues supplied by this artery has been shown to increase by about 30% (Wilson 1981).

It is unlikely that portal venous pressure is greatly changed by perfusion of the superior mesenteric artery at different rates since Bower & Groszman (1982) have reported a very low mean slope resistance in the portal vein of anaesthetized cats such that only small changes in portal venous pressure result from large changes in induced portal venous flow. If mean arterial pressure and hepatic portal venous pressure are unchanged, as reported here to be the case in animals with an intact splanchnic circulation, then a 30% increase in flow requires a 30% fall in resistance, considerably more than we have found.

Unfortunately, in our experiments, we were unable to approach flow rates equivalent to the 5–10 ml min^{-1} occuring physiologically in the superior mesenteric artery of the rat (Wilson 1981; Hiley, unpublished observations); they produce unacceptable degrees of haemolysis and marked disturbances of central arterial pressure. It is possible that, if these higher flow rates had been achieved, there would have been divergence of the pressure/flow relations and a greater fall in resistance to flow observed. Thus, pretreatment with either the barbiturate or the glucocorticoid may have modified the structure of the mesenteric arterial bed as a result of, or in order to give rise to, the increased blood flow, and hence the distensibility of the vessels may change. Such an effect might be more prominent in pressure/flow relations nearer the physiological pressures experienced by the superior mesenteric artery and thus result in greater reductions in resistance.

The experiments with the perfused hindquarter were carried out in order to assess whether the reduced resistance was related to the observed flow effects in the intact hepatosplanchnic circulation and not the result of a body-wide change in vascular smooth muscle. This bed was chosen since it is not a regions like the brain and kidneys, which is capable of autoregulation and it might, therefore, provide a source for that part of the cardiac output diverted to the hepatosplanchnic bed by pretreatment with the compounds under investigation. These results indicate that the reductions of resistance in the superior mesenteric arterial bed are not common to the remainder of the vasculature.

There is a marked difference in the control values for the regression lines describing mesenteric arterial perfusion in the two experiments shown in Fig. 1 whereas there is good agreement for the control values in the hindquarter perfusion. The experiments in the mesentery were carried out some 8 months apart whereas the hindquarter experiments were performed in the same month. Thus there may be some seasonal variation in relative vascular resistance in the mesenteric arterial bed. This is partially supported by our previous observations that there may be considerable differences between batches of animals obtained at different times of the year in the percentage distribution of cardiac output to the hepatosplanchnic bed in saline-pretreated animals (Berman et al 1983).

The cause of the enhanced liver blood flow produced by phenobarbitone pretreatment remains unknown. Since neither central arterial nor portal venous pressure were changed by pretreatment with the barbiturate, the reduced perfusion pressure in the superior mesenteric bed is not, as had been previously postulated (Nies et al 1976; Yates et al

1978), the result of the myogenic arteriolar response reported by Johnson (1959) to take place in response to changes in hepatic portal venous pressure. It is also not the result of the known actions of phenobarbitone as a ganglion blocker or on vascular smooth muscle since it is less potent in these respects than amylobarbitone (Exley 1954; Altura & Altura 1975) which has an insignificant effect on liver blood flow (Yates et al 1978). Since the degree of enhancement of liver blood flow is of similar magnitude to the hepatic enlargement produced at several doses (Yates et al 1978) it remains likely that the liver itself procures the enhanced proportion of cardiac output, perhaps by means including local hormonal and neuronal mechanisms.

In the case of the glucocorticoid, it has been suggested that the increased intestinal blood flow is the result of enhanced water absorption (Donowitz et al 1979). Those authors reported a 43% increase in ileal blood flow in rabbits following 3 days pretreatment with 30 mg kg^{-1} daily of 6-methylprednisolone acetate and intestinal water uptake was found to be enhanced by 70–80%. It is, nevertheless, possible that both phenobarbitone and 6-methylprednisolone may act by means of a similar mechanism such as stimulation of the production of glucagon, a hormone that has been reported to bring about an increase in the blood flow through the hepatosplanchnic bed (Ohnhaus 1972).

In conclusion, the experiments reported here show that the increased hepatosplanchnic flow brought about by both phenobarbitone and 6-methylprednisolone are associated with reductions in resistance to perfusion of the superior mesenteric arterial bed with blood. However, the apparent reductions in resistance which are observed are somewhat smaller than would be predicted from the observed magnitude of the blood flow responses to pretreatment with these agents. It was further observed that there was no change in hepatic portal venous or central arterial pressure and no significant change in resistance in the iliac arterial bed.

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