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Influence of phenobarbital on changes in Na^+ handling, hemodynamics and liver function due to partial portal vein ligation in rats

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Abstract

This study examined the influence of phenobarbital, an inducer of hepatic enzymes, on Na^+ handling, hemodynamics and liver function (measured by the rate constant of elimination of aminopyrine in the aminopyrine breath test) after partial portal vein ligation. Rats were randomized to drink either phenobarbital + water or water only for 10 days and then underwent either sham operation or partial portal vein ligation. The aminopyrine rate constant of elimination and Na^+ balance were measured daily before and after surgery; after surgery, hemodynamic measurements were obtained daily in a subset of rats. Phenobarbital raised the baseline aminopyrine rate constant of elimination. Partial portal vein ligation, but not sham operation, caused equivalent reductions in the aminopyrine rate constant of elimination in phenobarbital- and water-treated groups, such that the aminopyrine rate constant of elimination remained higher in the former. Na^+ balance increased significantly in partial portal vein ligation + water, but not sham + water rats on day 1 and then decreased on days 2 and 3. In contrast, neither sham + phenobarbital nor partial portal vein ligation + phenobarbital rats had a significant increase in Na^+ balance. Partial portal vein ligation resulted in vasodilation on day 3 after surgery in the water-treated rats, an effect that was prevented by treatment with phenobarbital. These results support previous suggestions that a reduction in liver function triggers renal Na^+ retention in this model. Vasodilation is not necessary for the latter effect, but also appears to be dependent on a reduction in liver function.

Keywords: Portal hypertension; Na^+ retention; Liver function; Aminopyrine; Phenobarbital; Hemodynamics; Vasodilation

1. Introduction

The model of partial portal vein ligation in rats has been used extensively to examine the hemodynamic changes associated with portal hypertension and cirrhosis of the liver. It has been repeatedly demonstrated that, as in cirrhosis, partial portal vein ligation is associated with peripheral vasodilation, increased cardiac output, Na^+ retention and expansion of extracellular fluid volume, and porto-systemic shunting (Vorobioff et al., 1983; Colombato et al, 1991; Ohno et al., 1993). Some studies that examined the temporal relationship between Na^+ retention and the hemodynamic changes provided evidence to support the peripheral vasodilation hypothesis of Na^+ retention and ascites formation in cirrhosis (Colombato et al., 1991; Albillos et al., 1992). Others have noted a dissociation between the two events, suggesting that other factors may contribute to the Na^+ retention (Murakami et al., 1996).

In addition to the hemodynamic and renal functional changes mentioned above, partial portal vein ligation is characterized by a transient reduction in the metabolic activity of the liver, measured with the aminopyrine breath test and reflected by the aminopyrine rate constant of elimination (Ohno et al., 1993). A close association was detected between the magnitude of the reduction in the aminopyrine rate constant of elimination and the development of Na^+ retention (which was also transient and limited), irrespective of the hemodynamic changes, suggesting that reduced liver function may be the trigger for Na^+ retention in this model (Ohno et al., 1993; Murakami et al., 1996). This relationship between the reduction in the aminopyrine rate constant of elimination and the development of Na^+ retention had been previously described in other models of liver dysfunction, including two models of cirrhosis induced by carbon tetrachloride administration

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and by bile duct ligation, and a model of two-thirds partial hepatectomy (Wensing and Branch, 1990; Wensing et al., 1990; Ohno et al., 1991). In rats subjected to bile duct ligation, Na^+ retention developed approximately 2.5 weeks after surgery in association with a significant fall in the aminopyrine rate constant of elimination of 37 % from baseline. It was interesting to note that pretreatment with phenobarbital, an inducer of hepatic enzymes, raised the baseline aminopyrine rate constant of elimination by 45%, and delayed the development of Na^+ retention to 4 weeks after surgery. At this time, the aminopyrine rate constant of elimination had decreased by 57% from baseline, to reach a value similar to that of nontreated rats at 2.5 weeks after bile duct ligation. It appeared, therefore, that a reduction in liver oxidative function to a critical level was necessary before Na^+ retention became apparent, and that by enhancing oxidative metabolism, phenobarbital delayed the onset of Na^+ retention.

The above correlations between liver function and Na^+ handling, however, do not necessarily indicate a causal relationship between them. The purpose of this study was to provide more direct evidence to support such a causal relationship between the reduction in liver function and Na^+ retention. Using the partial portal vein ligation model, where the reduction in the aminopyrine rate constant of elimination is transient and not progressive as in cirrhosis, we hypothesized that treatment with phenobarbital of rats subjected to partial portal vein ligation would prevent the development of Na^+ retention, provided that the aminopyrine rate constant of elimination was not excessively decreased. Furthermore, concomitant measurement of renal and systemic hemodynamics would allow assessment of the relationship between the reduction in liver function, the altered renal Na^+ handling, and the hemodynamic changes induced by partial portal vein ligation, which may shed light on the pathogenesis of Na^+ retention in portal hypertension and, possibly, cirrhosis.

2. Materials and methods

2.1. General design

This study was approved by the Institutional Review Board-Animal Care Committee, American University of Beirut. Male Sprague–Dawley rats weighing 220–300 g were used for this study. Starting 10 days prior to the day of surgery (day 0), rats were randomized to receive either phenobarbital sodium (0.035% providing 1.4 μmol/ml of Na^+) or its vehicle (distilled water) in drinking water ($n = 55$/group), and this treatment was maintained until the end of the study. Rats were fed on regular rat chow until 3 days prior to surgery, when they were placed in metabolic cages, shifted to a low Na^+ rat chow (0.05%), and started on a constant daily Na^+ load (2.22 mEq of Na^+ per day) administered by oro-gastric gavage as a 1% solution of NaCl. This treatment was continued until the end of the study, and the rats remained in metabolic cages throughout this period. On the day of surgery, rats were randomized to undergo either sham operation or partial portal vein ligation ($n = 24$/group). One day prior to surgery, and on days, 1, 2 and 3 after surgery, the aminopyrine rate constant of elimination and Na^+ balance were measured. A subset of rats ($n = 7$/group) underwent hemodynamic studies on the day preceding surgery, i.e., day (-1). The rest of the rats underwent hemodynamic studies on either day 1, 2 or 3. Thus, of the rats which underwent surgery on the portal vein, there were four major groups: sham-operated rats treated with either water or phenobarbital (sham + water and sham + phenobarbital, respectively) and rats with partial portal vein ligation similarly treated (partial portal vein ligation + water and partial portal vein ligation + phenobarbital). Furthermore, in each of the four groups, there were three subgroups ($n = 8$ in each), depending on the day hemodynamic studies were conducted (day 1, 2 or 3).

2.2. Surgical procedure

Sham operation and partial portal vein ligation were performed as previously described (Ohno et al., 1993). Briefly, under ether anesthesia, an incision was made in the abdomen. The portal vein was identified, a 20-gauge blunt-tipped needle was placed along side it in parallel, and a suture was tied around both. The needle was then removed and the portal vein was allowed to expand to the limits of the ligature. The abdominal incision was then double sutured. Sham-operated rats underwent the same procedure, but no ligature was tied around the portal vein.

2.3. Measurement of the aminopyrine rate constant of elimination and Na^+ balance

The aminopyrine rate constant of elimination was measured as previously described (Wensing et al., 1990). Briefly, rats received 0.15 μCi of [^{14}C]Aminopyrine (Amersham International, England) intraperitoneally and were placed in glass cages with an air inlet valve and an exit valve connected to a vacuum. Expired air was passed through concentrated sulfuric acid and was collected in scintillation vials containing a 1:4 mixture of ethanolamine:methanol. Samples were obtained over 15-min periods for 2 h. Radioactivity was measured in a liquid scintillation counter. The aminopyrine rate constant of elimination was calculated using least-squares regression analysis of the logarithm of the counts vs. time.

Starting on day (-1) and continuing till the end of the study, rats were weighed daily and food intake and urine volume over 24 h were measured. Na^+ intake was calculated as the sum of that given by gavage and that ingested in food; for the phenobarbital group Na^+ intake included that given as Na^+ phenobarbital. Concentrations of Na^+ in

urine were measured with ion-selective electrodes at the Chemistry Laboratory of the American University Hospital. Na^+ balance was calculated as the difference between daily Na^+ intake and urinary excretion.

2.4. Hemodynamic studies

On days (−1), 1, 2 and 3, subsets of rats underwent hemodynamic studies under pentobarbital anesthesia (50 mg/kg, intraperitoneally). A PE-205 tube was placed in the trachea to maintain airway patency. Two PE-50 catheters were placed in the right jugular vein for administration of fluids and [^{3}H]inulin. A thermistor probe was placed in the left carotid artery and advanced till the arch of the aorta. This probe was connected to a Cardiotherm cardiac output computer (Columbus Instruments International, Columbus, OH) for measurement of cardiac output by the thermodilution technique. At this time, three measurements of cardiac output were obtained at 5-min intervals. The average of these three readings represented the cardiac output of the rat and was used to calculate the cardiac index, expressed as ml/min/100 g of body weight. Immediately afterwards, the thermistor probe was removed and replaced by a PE-50 catheter connected to a pressure transducer and a Digimed blood pressure analyzer (Micro-Med, Louisville, KY) and systolic, diastolic and mean arterial pressures were continuously recorded. Peripheral vascular resistance was calculated as the mean arterial pressure/cardiac index. After that, a PE 190 catheter was introduced into the urinary bladder through the urethra for collection of urine samples. [^{3}H]inulin (dissolved in 0.9% saline) was then administered as a bolus dose of 0.75 μCi followed by a continuous infusion of 0.05 μCi/50 μl/min. 30 min later, urine collection was started for 30 min, at the midpoint of which an arterial blood sample was withdrawn. Glomerular filtration rate was calculated as the renal clearance of inulin.

2.5. Statistical analysis

Values are presented as means ± standard error of the mean. Comparison of values for the aminopyrine rate constant of elimination and Na^+ balance on different days in the same group was conducted using the paired Student's *t*-test for 2 days, and using repeated measures analysis of variance (ANOVA) for more than two values. If ANOVA revealed a significant difference, Dunnett's test was used for comparison of values on days 2 and 3 with the control values on day (−1). Comparison of values between different groups was conducted using the unpaired Student's *t*-test for two groups and ANOVA for more than two groups. If ANOVA showed a significant difference, multiple comparisons were conducted using the Student–Newman–Keuls test. For all tests, a *P*-value less than 0.05 was considered significant.

3. Results

3.1. The aminopyrine rate constant of elimination and Na^+ balance

In line with the methodology described above, there were 24 rats in each of the four major groups of rats with values for the aminopyrine rate constant of elimination and Na^+ balance on days −1 and 1, 16 rats with values extending to day 2, and only eight with values extending to day 3.

In sham-operated rats receiving water the aminopyrine rate constant of elimination (in $min^{-1} \times 10^{-2}$) did not change from day (−1) to days 1, 2 and 3 after surgery (from 2.04 ± 0.08 to 2.00 ± 0.08 to 2.02 ± 0.12 to 2.13 ± 0.18, respectively, Fig. 1). Na^+ balance (mEq/day) in these rats did not change on day 1 (from 1.36 ± 0.13 to 1.38 ± 0.10), but decreased significantly on days 2 and 3 after surgery (to 0.55 ± 0.12 and 0.76 ± 0.16, respectively). In contrast, in partial portal vein ligation rats receiving water, there was a significant reduction in the aminopyrine rate constant of elimination on day 1 after surgery (from 2.05 ± 0.07 to $1.51 \pm 0.13 \times 10^{-2}$ min^{-1}, $P < 0.0001$) accompanied by a significant rise in Na^+ balance (from 1.40 ± 0.11 to 1.98 ± 0.04 mEq/day, $P < 0.0001$) (Fig. 1). On day 2 after surgery, the aminopyrine rate constant

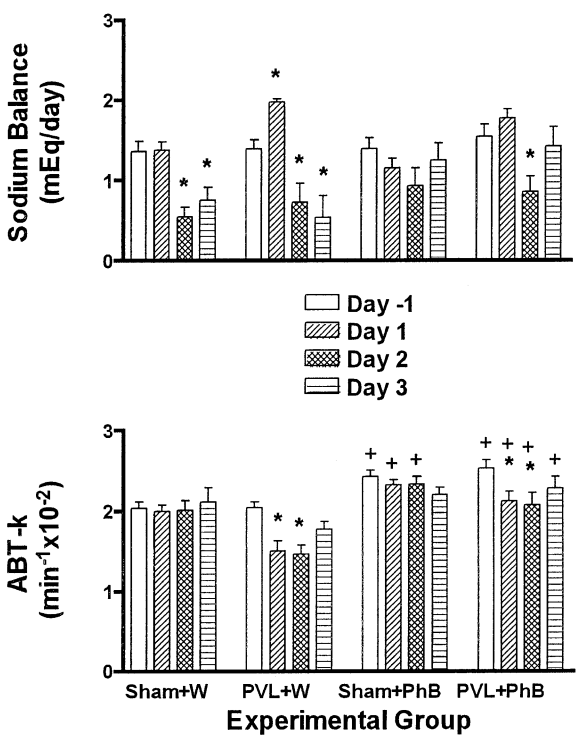

Fig. 1. The aminopyrine rate constant of elimination (ABT-K) and Na^+ balance 1 day before (Day-1, $n = 24$) and on days 1 ($n = 24$), 2 ($n = 16$) and 3 ($n = 8$) after partial portal vein ligation (PVL) or sham operation (Sham) in water (W)- or phenobarbital (PhB)-treated rats. *, $P < 0.05$ compared with Day (−1). +, $P < 0.05$ compared with the corresponding water-treated group on the same day.

of elimination remained depressed ($1.47 \pm 0.11 \times 10^{-2}$ min^{-1}) and Na^+ balance was reduced in partial portal vein ligation rats to a value similar to that observed in sham-operated rats on that day (0.73 ± 0.22 mEq/day). In the eight rats which were studied up to day 3 after surgery, there was an increase in the aminopyrine rate constant of elimination to a value ($1.78 \pm 0.09 \times 10^{-2}$ min^{-1}, $n = 8$) not significantly different from the baseline (day -1) value. Na^+ balance remained as low as on day 2 (0.54 ± 0.27 mEq/day).

Rats treated with phenobarbital had a significantly higher aminopyrine rate constant of elimination before surgery compared with water-treated rats, but a similar Na^+ balance (Fig. 1). In sham-operated rats, the aminopyrine rate constant of elimination (in $min^{-1} \times 10^{-2}$) did not change throughout the study (from 2.43 ± 0.10 on day (-1) to 2.33 ± 0.06 on day 1, 2.34 ± 0.09 on day 2 and 2.21 ± 0.09 on day 3, Fig. 1). Na^+ balance did not change from baseline on days 1 (from 1.40 ± 0.13 to 1.16 ± 0.12 mEq/day), 2 (0.93 ± 0.23 mEq/day) or 3 (1.25 ± 0.22 mEq/day) after surgery. In phenobarbital-treated rats with partial portal vein ligation, the aminopyrine rate constant of elimination decreased significantly from day (-1) ($2.54 \pm 0.10 \times 10^{-2}$ min^{-1}) to days 1 and 2 (2.13 ± 0.12 and $2.08 \pm 0.15 \times 10^{-2}$ min^{-1}, respectively), and recovered on day 3 to a value not significantly different from the day (-1) value ($2.29 \pm 0.14 \times 10^{-2}$ min^{-1}). At all times, however, the aminopyrine rate constant of elimination remained significantly higher than the value in water-treated rats with partial portal vein ligation on corresponding days, and was similar to the value obtained on day (-1) in those rats. In association with the decrease in the aminopyrine rate constant of elimination, there was a slight, but not significant, increase in Na^+ balance in phenobarbital-treated rats with partial portal vein ligation on day 1 (from 1.55 ± 0.16 to 1.78 ± 0.11 mEq/day, $P = 0.1$) (Fig. 1). On day 2 after surgery, there was a significant decrease in Na^+ balance (to 0.86 ± 0.19 mEq/day). Rats studied on day 3 had a Na^+ balance similar to the baseline value (1.43 ± 0.24 mEq/day).

3.2. Hemodynamic studies

There were no significant differences in mean arterial pressure, cardiac index, or glomerular filtration rate between sham-operated rats receiving water and those receiving phenobarbital (Table 1, Figs. 2 and 3), indicating that phenobarbital treatment per se did not influence these parameters. In contrast, peripheral vascular resistance was lower in the phenobarbital treated rats compared with the water treated rats (3.44 ± 0.16 vs. 4.21 ± 0.33, $P = 0.05$).

There was no significant change in mean arterial pressure over time in any of the groups studied. Furthermore, mean arterial pressure was not different between sham-operated and partial portal vein ligation rats on any day in either the water- or the phenobarbital-treated groups (Table 1).

Table 1
Mean arterial pressure and glomerular filtration rate on days (-1), 1, 2 and 3 after surgery in rats treated with water or phenobarbital which underwent sham operation (Sham) or partial portal vein ligation (PVL)

	Water-treated		Phenobarbital-treated	
Day	Sham	PVL	Sham	PVL
Mean arterial pressure (mmHg)				
Day (-1)	115 ± 9 (6)		105 ± 5 (7)	
1	115 ± 6	109 ± 7	111 ± 7	103 ± 9
2	123 ± 5	105 ± 8	109 ± 8	104 ± 6
3	120 ± 2	110 ± 6	123 ± 5	125 ± 5
Glomerular filtration rate (ml / min / 100 g)				
Day (-1)	1.43 ± 0.29 (6)		1.26 ± 0.33 (6)	
1	0.74 ± 0.14	0.66 ± 0.12	0.79 ± 0.16	0.69 ± 0.15 (7)
2	1.07 ± 0.17 (7)	0.91 ± 0.18	0.57 ± 0.15	0.58 ± 0.20
3	0.58 ± 0.23 (7)	0.47 ± 0.18 (7)	0.79 ± 0.16 (7)	1.09 ± 0.28 (7)

Values are means ± standard error of the mean. Numbers in parentheses refer to number of animals; where not indicated $n = 8$. No significant differences were found between day (-1) values and those on other days in any group, or between values in partial portal vein ligation and sham-operated rats on similar days post-surgery in either group.

Cardiac index (in ml/min/100 g) increased significantly in both sham + water and partial portal vein ligation + water groups on day 1 (41 ± 3 and 42 ± 5, respectively) relative to day (-1) (28 ± 2) (Fig. 2). However, it returned to the presurgery level in the sham + water group on days 2 (35 ± 4) and 3 (32 ± 2), while it was sustained in the partial portal vein ligation + water (42 ± 5 and 43 ± 4). Comparison of the cardiac index between the sham + water and partial portal vein ligation + water groups on days 1, 2 and 3 revealed similar values on days 1 and 2, and a significantly higher value in the partial portal vein ligation + water group on day 3.

In contrast to the above, the cardiac index did not increase in either the sham + phenobarbital or partial portal vein ligation + phenobarbital groups on days 1 (34 ± 1 and 39 ± 4, respectively) and 2 (33 ± 2 and 41 ± 4, respectively) after surgery relative to day (-1) (31 ± 2). On day 3, the cardiac index was not different in the sham + phenobarbital (30 ± 5) group compared to day (-1). In contrast, the cardiac index on day 3 was higher in the partial portal vein ligation + phenobarbital (43 ± 3) group compared to that on day (-1). In addition, comparison of the cardiac index between the sham + phenobarbital and partial portal vein ligation + phenobarbital groups on days 1, 2 and 3 revealed similar values on days 1 and 2, and a significantly higher value in the partial portal vein ligation + phenobarbital compared to the sham + phenobarbital only on day 3.

Peripheral vascular resistance (mmHg min 100 g/ml) decreased significantly in both the sham + water and the partial portal vein ligation + water groups on day 1 (2.94 ± 0.29 and 2.76 ± 0.29, respectively) relative to day (-1) (4.21 ± 0.33) (Fig. 3). However, it returned to the presurgery level in the sham + water group on days 2

(3.80 ± 0.46) and 3 (3.93 ± 0.26), while it remained low in the partial portal vein ligation + water group on both these days (2.66 ± 0.31 and 2.70 ± 0.18, respectively). Comparison of peripheral vascular resistance between the sham + water and partial portal vein ligation + water groups on days 1, 2 and 3 revealed similar values on days 1 and 2, and a significantly lower peripheral vascular resistance in the partial portal vein ligation + water group on day 3.

In contrast, in the phenobarbital-treated groups, no differences in peripheral vascular resistance were noted on day (−1) (3.44 ± 0.16) and days 1, 2 and 3 in either the sham + phenobarbital or the partial portal vein ligation + phenobarbital groups. In addition, no significant differences were observed between the two groups on any particular day after surgery (sham vs. partial portal vein ligation: day 1: 3.33 ± 0.25 vs. 2.86 ± 0.47, day 2: 3.28 ± 0.13 vs. 2.71 ± 0.25, day 3: 3.58 ± 0.21 vs. 3.03 ± 0.24, respectively).

There were no significant differences in glomerular filtration rate over time, or between sham-operated and partial portal vein ligation rats, in either the water- or phenobarbital-treated groups, or between phenobarbital- and water-treated rats in either the sham-operated or partial portal vein ligation groups, on any day after surgery (Table 1).

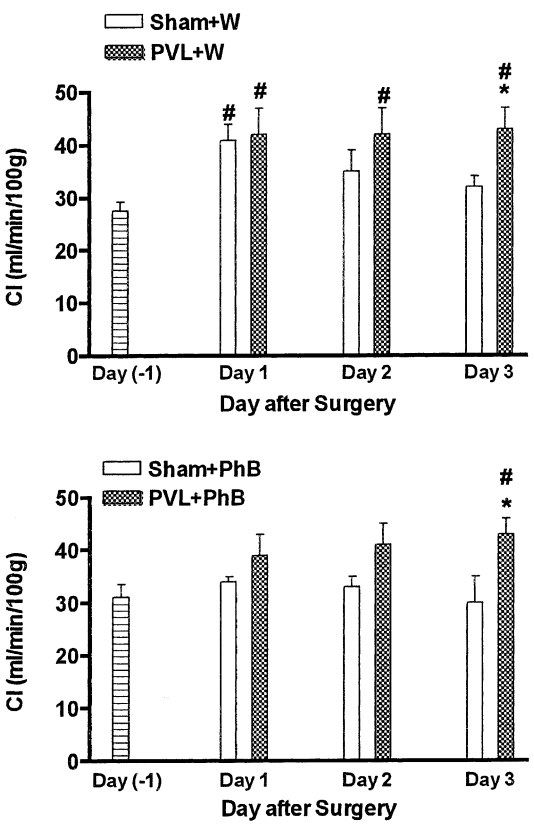

Fig. 2. Cardiac index (CI) in rats treated with either water (W) or phenobarbital (PhB) on days 1, 2 or 3 after either sham operation (Sham) or partial portal vein ligation (PVL). $n = 8$/group/day. *, $P < 0.05$ compared with sham-operated group receiving similar treatment (W or PhB). #, $P < 0.05$ compared with Day (−1).

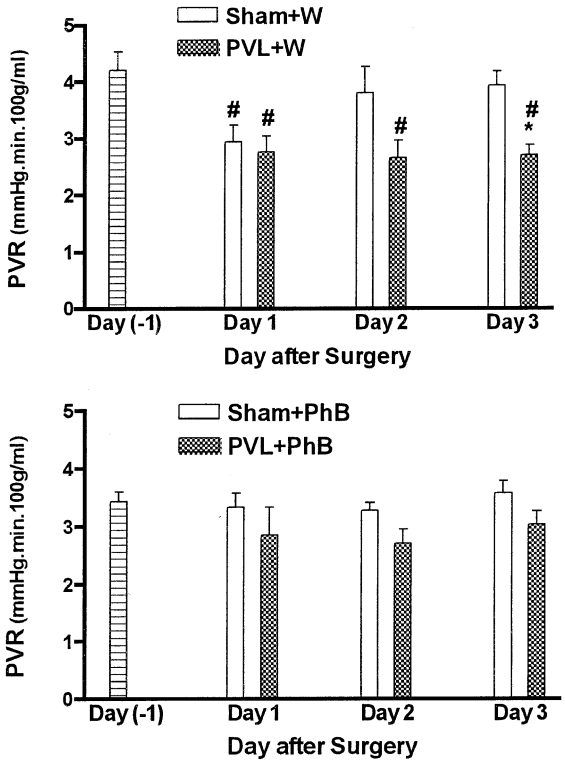

Fig. 3. Peripheral vascular resistance (PVR) in rats treated with either water (W) or phenobarbital (PhB) on days 1, 2 or 3 after either sham operation (Sham) or partial portal vein ligation (PVL). $n = 8$/group/day. *: $P < 0.002$ compared with Sham + water on Day 3. #: $P < 0.05$ compared with Day (−1).

4. Discussion

This study reveals that pretreatment with phenobarbital influences the extent of the reduction in liver function, the development of Na^+ retention and the extent of the hemodynamic changes due to partial portal vein ligation. The results provide more direct evidence supporting an important role for a reduction in liver function in controlling and triggering abnormal renal handling of Na^+ during liver dysfunction due to partial portal vein ligation, and possibly, cirrhosis.

Previous studies with the same model have clearly shown that Na^+ retention by the kidney is an early event, occurring in most cases on day 1 after partial portal vein ligation (Ohno et al., 1993; Murakami et al., 1995, 1996). These studies also show that the reduction in liver function is most severe on days 1 and 2 after surgery and this is followed by recovery of function toward baseline. The hyperdynamic circulation, with a reduction in vascular resistance and an increase in cardiac output, also develops

early and is established by 3 or 4 days after partial portal vein ligation, with no change in these parameters or in plasma volume or Na^+ space at later stages (Colombato et al., 1991; Albillos et al., 1992). Therefore, in order to determine the interrelationship among the changes in hemodynamics, liver function and Na^+ handling, and to evaluate the influence of phenobarbital on them, it is essential to conduct studies at the time these changes are ongoing, which is what was done in the present study.

Consistent with the results of previous studies, there was a clear rise in Na^+ balance on day 1 after partial portal vein ligation in rats receiving water. This finding, in the absence of other obvious routes of Na^+ loss, suggests the development of Na^+ retention by the kidney. Sodium retention in this model preceded the development of peripheral vasodilation, was associated with a significant reduction in the aminopyrine rate constant of elimination, and was not accompanied by an alteration in the glomerular filtration rate. These results, therefore, suggest that peripheral vasodilation per se is not the trigger for Na^+ retention. More likely, a factor which escaped the metabolic activity of the liver enhanced tubular Na^+ reabsorption by a direct action on tubular cells and not by diminishing the filtration of solute. These conclusions, although in an acute model of liver dysfunction, are reminiscent of the "overflow" hypothesis of Na^+ retention and ascites formation in cirrhosis (Lieberman et al., 1970). It should be noted that both sham-operated and partial portal vein ligation rats had a significant decrease in Na^+ balance on day 2 after surgery, despite the fact that partial portal vein ligation rats had a sustained reduction in the aminopyrine rate constant of elimination. This suggests that there are factors influencing Na^+ handling in these rats other than partial portal vein ligation, and that these factors may relate to surgery and anesthesia per se.

Further support for these conclusions is obtained from the results of the phenobarbital-treated groups. The fact that the baseline aminopyrine rate constant of elimination was higher in these groups attests to the adequacy of phenobarbital treatment in inducing the hepatic enzymes. In these groups, there was no change in Na^+ balance from day 0 to day 1 after partial portal vein ligation by paired analysis in 24 rats. Thus, phenobarbital prevented the development of Na^+ retention in this model. The only associated influence of phenobarbital treatment was on the aminopyrine rate constant of elimination, which was enhanced at baseline and which, although significantly reduced on day 1, remained higher than corresponding values in water-treated rats, and close to their baseline values.

The hemodynamic changes induced by partial portal vein ligation were also affected by treatment with phenobarbital. In the water-treated groups, it appeared that surgery per se caused a reduction in peripheral vascular resistance and an increase in cardiac index on day 1. The observation that only the partial portal vein ligation group developed Na^+ retention suggests that the vasodilation was not the trigger for it because vasodilation occurred in both partial portal vein ligation and sham-operated groups to the same extent. By day 2, sham-operated rats had regained their baseline peripheral vascular resistance and cardiac index. At this time, the effect of partial portal vein ligation became apparent with maintenance of the observed decrease in peripheral vascular resistance and increase in cardiac index, which became clearly significant on day 3. In contrast to this course, these parameters did not change in phenobarbital-treated rats over time, nor between groups, except for a rise in cardiac index on day 3. Thus, phenobarbital prevented the development of vasodilation due to partial portal vein ligation. The absence of vasodilation in phenobarbital-treated rats suggests a role for reduced liver function in triggering vasodilation in this model of portal hypertension, although it may not be the sole cause. This is reminiscent of the "peripheral arterial vasodilation hypothesis," which suggests that vasodilation may result from altered liver function (Schreier et al., 1988).

It is interesting to note that the absolute decrease in the aminopyrine rate constant of elimination in both water- and phenobarbital-treated rats was similar (0.55 ± 0.11 vs. 0.41 ± 0.08 min^{-1} on day 1, $P = 0.28$), suggesting that the magnitude of the insult to the liver was similar. The difference was in the absolute value of the aminopyrine rate constant of elimination that was reached after partial portal vein ligation. Thus, it appears that the aminopyrine rate constant of elimination has to fall to a critical level before Na^+ retention becomes manifest. These results suggest either that it is enzyme activity itself, which is the hepatic determinant of renal Na^+ handling, or that enzyme induction by phenobarbital influences the hepatic determinant of renal function to an extent similar to its influence on oxidative metabolism.

Previous studies have used the partial portal vein ligation model to investigate the events that occur in cirrhosis, based upon the many similarities mentioned in the Introduction. This, however, may not be justifiable since the partial portal vein ligation model is one of acute and transient changes in liver function and renal Na^+ handling. Within 4 days, the hyperdynamic circulation is established and active Na^+ retention is no longer apparent (Sikuler et al., 1985; Ohno et al., 1993; Murakami et al., 1996). Furthermore, there are numerous changes in cirrhosis (including the progressive nature of the disease and destruction of liver parenchyma, activation of neuro-humoral mechanisms, the presence of ascites, endotoxemia, etc.) that are different from, or more severe than, those observed in the partial portal vein ligation model. Nonetheless, it may be worthwhile to compare our results for this model with those obtained by others. Based upon the finding that a decrease in peripheral vascular resistance preceded the expansion of both extracellular volume (Colombato et al., 1991) and of Na^+ space, as measured by the volume of distribution of ^{22}Na (Albillos et al., 1992), those

studies have suggested that vasodilation is a sufficient stimulus for the development of Na^+ retention in cirrhosis. These results appear contradictory to the results of the present study. There are two explanations for the discrepancy: one is that vasodilation was detected on day 1 in those studies, while in the present study it occurred later. We have no clear explanation for this, but it should be noted that earlier studies revealed that the point at which a decrease in splanchnic resistance and an increase in portal venous inflow became clearly established was on day 4 after partial portal vein ligation (Sikuler et al., 1985). The second is that Na^+ retention was detected on day 2, while in our present and previous studies (Murakami et al., 1996), Na^+ retention was detected as early as day 1 after partial portal vein ligation. The reason for this discrepancy relates to methodological differences. In our studies, Na^+ retention was assessed by administering a NaCl load, measuring renal excretion of Na^+, and calculating Na^+ balance. Thus, this method directly assessed the renal handling of Na^+ ions at the time it was conducted. In addition, the measurement of glomerular filtration rate indicated that the reduced Na^+ excretion was not a result of impaired filtration of Na^+, but of enhanced tubular reabsorption. In contrast, the other studies did not measure the glomerular filtration rate nor the renal handling of Na^+, but demonstrated an increased Na^+ space, a parameter which indicates that Na^+ retention by the kidney has occurred at an earlier time, but it does not reflect the renal handling of Na^+ at the time the study is conducted. Furthermore, in the present study Na^+ intake was maintained almost constant by daily gavage, while in the other studies rats were allowed free access to rat chow. It has been previously demonstrated that food intake is markedly depressed during the first few days after partial portal vein ligation (Halverson and Myking, 1979), a finding confirmed in the present study where measurement of food intake (g/day) before and after partial portal vein ligation revealed a decrease from 24.0 ± 1.2 on day (-1) to 3.6 ± 0.6 on day 1, 11.3 ± 1.3 on day 2 and a rise to 24.9 ± 13.2 on day 3. This represents a proportional decrease in Na^+ intake. It is clear, therefore, that rats whose only source of Na^+ is through food will effectively have a restriction of Na^+ intake during the first few days after surgery. This will mask the development of renal Na^+ retention and delay its manifestation, particularly when expansion of Na^+ space is used to assess Na^+ status, and when the renal excretion of Na^+ ions is not measured directly. Therefore, those studies that use Na^+ space as an index of Na^+ retention have to ensure a constant Na^+ intake over the course of the study, otherwise the conclusions may be questionable. Hence, not only is extrapolation from portal vein ligation to cirrhosis questionable, but the results provided earlier on the correct chronological sequence of events may have to be reassessed.

The model used in the present study is an acute model of portal hypertension in which a vein carrying approximately 25% of the cardiac output is constricted. It is conceivable that this constriction, per se, will lead to edema formation in the splanchnic area and to some degree of intravascular volume depletion that is sensed by the baroreceptors to stimulate compensatory mechanisms. This sequence of events may indeed contribute to the Na^+ retention that is observed early after portal vein ligation, but it does not explain the marked inhibition of Na^+ retention by phenobarbital because the same surgical approach and extent of portal vein narrowing was used in all rats.

The purpose of the present study was to explore the relationship between a reduction in liver function and renal Na^+ retention, which was previously described in cirrhosis and other models of liver dysfunction. We provide evidence to support such a partial dependence of Na^+ handling on liver function, and the ability to manipulate it with a pharmacological intervention. If, as we hypothesize, this relationship holds in cirrhosis, as human studies have also suggested (Wood et al., 1988; Wensing et al., 1997), it might explain the onset of Na^+ retention in the course of the disease although it may not be the sole mechanism. Based on this, the use of phenobarbital to delay the onset, and slow the progression, of Na^+ retention and ensuing complications in cirrhosis warrants further study.

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