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MODIFICATION OF PULMONARY METABOLISM OF NORADRENALINE IN EXPERIMENTAL OBSTRUCTIVE JAUNDICE

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Abstract—The pulmonary metabolism of noradrenaline (NA) was measured in lungs removed from 3 day sham-operated rats and from rats whose bile ducts had been ligated 3 days earlier (BDL). The pulmonary metabolism of NA as measured by a single clearance of the radio-labelled ^{14}C-amine was significantly increased in lungs excised from BDL rats as compared to that measured in the sham-operated rats. The change in metabolism was associated with an alteration in the pulmonary uptake of NA and not with the activities of the enzymes monoamine oxidase types A and B and catechol-*O*-methyl transferase. Moreover, it was not correlated with rises in the bilirubin or cholesterol concentrations in the serum of the BDL rats and occurred independent of any changes in pulmonary pressure. In a second series of experiments, the evolution of this abnormality over the period of one to six days postoperative was investigated. In the sham-operated rats, there was no significant change in the pulmonary metabolism of NA even by the sixth day. In contrast, there were time-dependent increases from one to six days in these metabolic processes in BDL rats with the highest values being at six days. In contrast, the serum concentrations of bilirubin and cholesterol and activities of the enzymes, alanine transaminase and alkaline phosphatase all rose to their maximum by the fourth day and thereafter declined. Although serum albumin levels fell significantly in BDL rats they were not significantly different from sham-controls. Thus, change in pulmonary metabolism of NA with obstructive jaundice increases with time from one to six days and it not related to the blood chemical changes of biliary obstruction or hepatic synthetic function.

Patients with cirrhosis and obstructive jaundice tend to be hypotensive and have reduced total peripheral resistance in conjunction with an increase in cardiac output or index [1, 2]. Moreover, cirrhotic patients have blunted pressor responses to intravenous infusions of noradrenaline (NA) and sympathetic stimulation, as opposed to adrenaline, suggesting a defect specific to NA [3]. In addition patients with obstructive jaundice are highly susceptible to post-operative shock [4]. Such vascular instability can be readily demonstrated in experimental animals with ligation of the bile duct (BDL) [5], which are widely used as models of both cirrhosis and obstructive jaundice.

The biochemical lesion of this vascular instability in such circumstances is unknown, but it has been postulated that it might be due to an alteration in peripheral neuroeffector mechanisms and in particular peripheral catecholamine metabolism [6].

The lung has been shown previously to be selective in its ability to metabolize amines by virtue of specific uptake processes [7]. For example, dopamine and tyramine are not metabolized by the rat lung as they are not taken up by the pulmonary endothelium. Serotonin (5-HT) is metabolized by monoamine oxidase (MAO) type A within the pulmonary endothelium cell [8, 9]. NA is metabolized intracellularly by MAO and catechol-*O*-methyl transferase (COMT) and the rate limiting step is the uptake process [10]. Non-hydroxylated, hydrophilic non-polar amines such as β-phenylethylamine (PEA) are avidly removed from the circulation by a process which is not active uptake and metabolised by MAO type B [11, 12].

Since blunted pressor responses to NA have been observed in both patients with cirrhosis and in animals whose bile ducts have been ligated, we decided to use the isolated rat lung perfusion technique in an attempt to assess whether or not altered pulmonary metabolism of NA could explain this observation.

MATERIALS AND METHODS

Materials

Unlabelled noradrenaline (NA), serotonin (5-HT), β-phenylethylamine (PEA) and *S*-adenosyl-L-methionine (SAM) of the highest purity were obtained from Sigma Chemical Corporation (U.S.A.). Radio-labelled ^{14}C-NA (norepinephrine hydrochloride 30–60 mCi/mmole), ^{14}C-5-HT (5-hydroxytryptamine creatinine sulphate 15–30 Ci/mmole), ^{14}C-PEA (phenylethylamine hydrochloride 40–60 mCi/mmole), ^{3}H-SAM (*S*-adenosyl-L-methionine 5–15 Ci/mmole) were purchased from New England Nuclear (Massachusetts, U.S.A.).

Animals

Sprague–Dawley male rats (≈200 g) were used for all the experiments. Experimental cholestasis (BDL) was induced by ligation of the bile duct three days

prior to the measurement of amine metabolism. In order to be included in the experiment, these animals had to have jaundiced extremities, bilirubinuria, jaundiced subcutaneous fat tissues as well as dilation of the bile duct proximal to the site of ligation. Blood was also collected from some of these animals for biochemical evaluation. Those animals that were not clinically jaundiced or had serum total bilirubin levels less than 34 μM/l were excluded from the study. Sham-operated animals underwent surgery at the same time, but their bile ducts were dissected free and not tied. In a second series of experiments, the metabolism of NA 1–6 days after either BDL or sham surgery was also measured. In this experiment, all the animals were weighed pre-operatively and then daily until the day of perfusion. In each rat, at the time of death, blood samples were taken to measure liver function by measuring serum total bilirubin, protein and albumin and cholesterol concentrations, and the activities of the serum alanine transferase (SGPT) and alkaline phosphatase. Blood was drawn from the heart of some of the jaundiced rats for bacterial cultre. Serum total bilirubin was measured by using the Jendrassik and Grof method [13] and direct spectrophotometry. Serum total protein was measured using the Lowry technique [14], and albumin by electrophoresis. The activity of the enzymes were determined colorimetrically using the procedure described by Reitman and Frankel [15] for SGPT and Lowry *et al.* [16] for alkaline phosphatase. Serum cholesterol was determined colorimetrically using the method outlined by Abell *et al.* [17].

Measurement of the metabolic activity of the lung

Perfusion studies. The total metabolism of NA was measured using the isolated lung perfusion technique [9]. Lungs from sham-operated and BDL rats were excised, weighed and perfused with warmed (37°) oxygenated Krebs' (pH 7.4) solution (in mmole/l NaCl 118; KCl 4.7; NaH_2PO_4 1.33; $NaHCO_3$ 25; $CaCl_2$ 2.7; $MgCl_2$ 1.05; glucose 5.56) at 8 ml/min. When the lungs were free of blood, a solution of radiolabelled 15 μM NA (cpm not less than 60,000/ml) in Krebs' solution was infused into the pulmonary artery at 0.4 ml/min for 3 min. The solution of NA was prepared by adding labelled NA to a 15 μM solution of unlabelled NA, prepared on the day of experimentation. This concentration of NA was chosen as the rate-limiting process in NA metabolism, namely uptake, has a K_m of about 1 μM [8]. During this period and for a further 5 min the effluent was collected as a single fraction. At the end of eight minutes the lungs were removed from the perfusion system, dissected free of all non-lung tissue, placed in cold (0°) 0.3 M perchloric acid and homogenised using two 10 sec bursts of a Ystral homogeniser Type X1020 at a setting of 8. The homogenate was centrifuged at 40,000 *g* for 10 min and the pellet discarded. The resultant supernatant was neutralized by careful addition of 1 M NaOH and samples of the neutralized supernatant as well as the samples of the lung effluent collected during the infusion were taken for chromatographic analysis using ion exchange chromatography. Amberlite CG 50, mesh 100–200, pH 6.3 columns were prepared as described by Tipton and Youdim [18]. Aliquots (100 μl) of collected lung perfusate and lung homogenate were passed across the columns, eluted with 2 ml of distilled water and the effluents collected. These effluents contained radiolabelled deaminated metabolites. Radioactivity was measured after mixing the samples in a toluene–triton scintillation fluid (0.5 g POPOP; 8 g PPO; 1 l. toluene; 1 l. triton X-100) using a liquid scintillation counter (Packard Tricarb B2450). Corrections were made for quenching using sample channels ratio. Using the resultant radioactive data of the collected lung perfusate and lung homogenates before and after their passage across the ion-exchange column, the pulmonary metabolism of the amine was calculated using the formula

$$\text{Total metabolism \%} = \frac{\text{dpm of metabolite in perfusate} + \text{dpm of metabolite in lung}}{\text{total dpm of perfusate} + \text{total dpm of lung homogenate}}$$

The percentage total metabolism of NA given in the 3-min infusion represents both uptake and deamination of NA on a single passage through the lung. This value was then converted to nM/g lung/min and the resultant figures used for statistical analysis of the data.

The measurement of amine uptake into lung tissue was identical to that previously described by Bakhle and Youdim [9]. Lungs from sham and BDL rats were perfused until free of blood (as described earlier). Radiolabelled 15 μM NA was infused for 3 min into the pulmonary artery at 0.4 ml/min. Thirty seconds after the completion of the infusion period, the lungs were removed from the system, homogenized in ice-cold (0°) 0.3 M perchloric acid and centrifuged at 40,000 *g* for 10 min. The resultant neutralized supernatant and effluent (3½-min collection) were then assayed for amine metabolites using ion exchange chromatography (as described previously). Uptake of NA was calculated from the total amount of radioactivity in the lung plus the radioactive deaminated metabolites in the collected effluent using the formula

$$\text{Uptake} = \frac{\text{dpm of lung homogenate} + \text{dpm of metabolite in collected lung perfusate}}{\text{dpm of infused } ^{14}\text{C-NA}}$$

and expressed as NA uptake in pM/g lung tissue/min. When the amount of metabolites is expressed as a proportion of the total amount of amine retained by the lung, this value has been shown to reflect the activity or activities of the enzymes in the lung. Using the radioactive counts of the lung homogenates before and after ion-exchange chromatography, this proportion was calculated

$$\text{Pulmonary enzyme activity \%} = \frac{\text{dpm of metabolite of lung}}{\text{total dpm within the lung}}$$

Radioactivity was measured in the same way as previously described.

In vitro *enzyme activities*

In vitro *MAO activity*. Pulmonary MAO type A and B activity was assessed by measuring the *in vitro* catabolism of 5-HT (type A) and PEA (type B) in lung homogenates prepared from sham-operated and BDL rats. The lungs were perfused until free of blood with warmed (37°) oxygenated Krebs' solution (pH 7.4) and then homogenized in ice-cold (0°) 0.32 M sucrose. Aliquots (50 μl) of lung homogenate containing about 0.2 mg protein were incubated with 50 μl of 1 mM ^{14}C-5HT or 20 μM ^{14}C-PEA each containing 25,000 cpm and 150 μl 0.1 M phosphate buffer pH 7.4 at 37° in a shaking water bath for 30 and 10 min respectively. These concentrations of substrates are saturating for MAO types A (5-HT) and B (PEA) [8]. At the end of the incubation period, the reaction was stopped by the addition of an enzyme inhibitor (10^{-3} M tranylcypromine) and the specimens assayed for deamination products using ion-exchange chromatography. The protein concentration of the homogenate was determined colorimetrically using bovine serum albumin as a standard [14]. Measurement of radioactivity in the homogenate was performed as described previously. The activities of MAO type A and B were calculated from the measured radioactivity after eluting the columns of radiolabelled deaminated metabolites and expressing the activity as nmol of deaminated metabolite generated per mg protein per minute.

In vitro *COMT activity*. Pulmonary COMT activity was measured in the same lung homogenates using radiolabelled SAM, as described by McCaman [19]. Aliquots (10 μl) of lung homogenate were incubated at 37° in a shaking water bath for 30 min with a 100 μl of substrate composed of 130 μl water, 30 μl 0.5 M phosphate buffer, 1 μl 1 M $MgCl_2$: 15 μl 50 mM dihydroxybenzoic acid in NaOH and 5 μl 2.94 mM ^{3}H-SAM. The reaction was stopped by adding 10 μl 3 N HCl and 400 μl ethylacetate was added. The tube was shaken in a vortex mixer and centrifuged at 3000 rpm. From the ethylacetate fraction, a 300 μl aliquot was removed, mixed with the toluene–triton scintillation fluid and counted. The activity of COMT was calculated from the amount of radioactivity present in the ethylacetate fraction and expressed as nmol/mg protein/min.

Pulmonary pressor response to NA

In some of the 3 day sham and BDL rats, the pressor responses to non-cumulative doses of NA were measured over the dose range 60 nm to 0.5 mM. This was done by connecting a Statham UC2 pressure transducer via a T-piece immediately proximal to the perfused lung. The output of the transducer was monitored on a Gould chart recorder.

Statistical analysis

All the data were statistically analysed using an unpaired Student's *t*-test. All results are presented as mean ± S.E.M. A P value of <0.05 was considered to be statistically significant for this study.

RESULTS

Table 1 describes the blood biochemical differences between the 3-day BDL and sham-operated rats in the first series of experiments. The serum from BDL rats had significantly higher bilirubin levels, alkaline phosphatase and transaminase activities than in the serum of blood taken from sham-operated rats.

In the second series of experiments involving the temporal changes in NA metabolism, the mean percentage weight loss in the sham-operated rats at 2 days post-operatively was 3.1 ± 3.4% and at 4 days 2.5 ± 2.0%. The weight loss at 6 days was 0.7 ± 0.6%. In contrast, ligation of the common bile duct caused a progressive loss of weight. The mean percentage weight loss after 2 days was 7.5 ± 1.5% and at 4 days, 12.5 ± 2.4%. At 6 days there was no further weight loss. Table 2 details the daily changes in the blood liver function tests following bile duct manipulation or ligation. In the BDL rats, bilirubin rose sharply one day after ligation and continued to rise to reach a peak by the fourth day. Thereafter, the concentrations fell, but remained significantly higher ($P < 0.05$) than the values seen in the sham-operated rats, which remained below the 34 μM/litre level. Cholesterol concentrations in the BDL rats followed a similar pattern to that of bilirubin but the peak occurred by the second day. Thereafter, concentrations fell to almost within the normal range by the sixth day. In the sham-operated rats, serum cholesterol values remained constant. The activities of the enzymes, alkaline phosphatase and SGPT in the BDL rats rose sharply after surgery to a peak between the first and second day. Thereafter the activities fell sharply. This post-operative pattern of changes of the activities of the enzymes was not observed in the sham-operated rats, although there

Table 1. The serum concentrations of bilirubin and the activities of alkaline phosphatase and alanine transaminase (SGPT) in 3 day sham-operated and BDL rats

	Sham-operated (N = 10)	BDL (N = 14)
Total bilirubin (μmole/l)	14 ± 3	197 ± 28*
Alkaline phosphatase (IU/l)	216 ± 53	346 ± 56†
SGPT (SF units/ml)	47 ± 6	190 ± 43*

Values shown are mean ± S.E.M. The numbers in brackets refer to the sample size.

* $P < 0.001$.

† $P < 0.025$.

Table 2. Daily changes in blood liver function tests following bile duct manipulation (sham) and ligation (BDL) following surgery

Days	Bilirubin (μmoles/l) Sham	Bilirubin (μmoles/l) BDL	Cholesterol (mg/dl) Sham	Cholesterol (mg/dl) BDL	Alkaline phosphatase (IU/l) Sham	Alkaline phosphatase (IU/l) BDL	SGPT (SF units/ml) Sham	SGPT (SF units/ml) BDL	Total protein (g/dl) Sham	Total protein (g/dl) BDL	Total albumin (g/dl) Sham	Total albumin (g/dl) BDL
1	10 ± 5	156 ± 43	57 ± 9	77 ± 8	165 ± 38	574 ± 77	51 ± 12	74 ± 29	—	—	—	—
2	12 ± 3	153 ± 3	75 ± 8	103 ± 13	288 ± 90	692 ± 172	54 ± 15	360 ± 85	—	—	—	—
3	17 ± 3	184 ± 26	63 ± 10	91 ± 16	164 ± 52	330 ± 106	61 ± 9	340 ± 95	5.1 ± 0.2	5.1 ± 0.2	2.6 ± 0.2	2.7 ± 0.8
4	22 ± 4	288 ± 65	64 ± 9	74 ± 13	120 ± 22	415 ± 107	50 ± 5	130 ± 47	—	—	—	—
5	7 ± 15	86 ± 35	56 ± 9	84 ± 21	250 ± 115	354 ± 81	48 ± 7	115 ± 36	—	—	—	—
6	15 ± 2	94 ± 36	56 ± 14	66 ± 16	200 ± 78	306 ± 80	47 ± 9	134 ± 19	5.5 ± 0.2	5.2 ± 0.4	2.5 ± 0.1	2.4 ± 1.0

Values shown are mean ± S.E.M. of 4–5 animals in each group.

were small increases in serum alkaline phosphatase activity. Over the same period, serum total protein concentrations did not alter whereas the albumin concentrations slowly fell in the sham-operated and BDL rats. This slow decrease in albumin concentrations appeared to be more rapid in the BDL rats as the concentrations at 6 days was significantly lower ($P < 0.02$) than at 3 days. In the sham-operated rats, the decrease was not significant.

NA metabolism in experimental obstructive jaundice: isolated perfused lung

The total metabolism of NA in 3-day BDL rats was significantly increased ($P < 0.05$) from 15.1 ± 1.7% in sham-operated rats to 22.8 ± 2.9%. This increase in metabolism of NA in a single passage through the rat lung is associated entirely with an increase in the uptake process for NA. The uptake of NA increased from 970 ± 166 pmole/g lung/min to 1910 ± 322 pmole/g lung/min ($P < 0.02$).

In the second series of experiments that examined the temporal changes, there was an initial fall in the metabolism of NA in the sham-operated rats from 28.3 ± 2.4% to 16.1 ± 1.2%, which recovered and returned to the immediate post-operative level of 28.4 ± 5.1% by the sixth day. On the other hand, BDL resulted in a time dependent increase in pulmonary metabolism rising from 30.0 ± 2.1% at day 1 to 45.9 ± 1.1% at day 6. The metabolism of NA measured in the lungs on the 2, 3, 5 and 6th days were significantly greater than the respective values obtained in the sham-operated rats (Fig. 1). In addition, the metabolism of NA at 6 days was significantly greater ($P < 0.001$) than its metabolism at 3 days.

When the metabolite in the lung was expressed as a proportion of the amount of radioactivity retained by the lung and used as an index of enzyme activity, no differences were found in this ratio in lungs removed from BDL or sham-operated rats. This finding indicated that the activities of catabolic enzymes are probably unaffected by BDL.

In vitro *measurement of pulmonary MAO and COMT activities*

Using saturating concentrations of 5-HT and PEA as specific substrates for MAO type A and type B

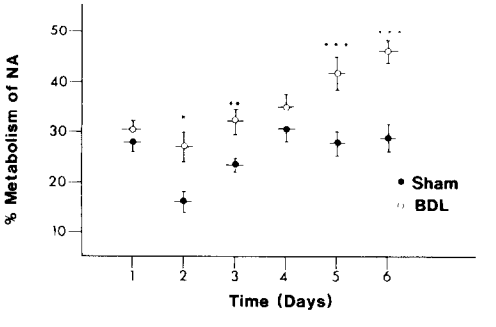

Fig. 1. The post-operative changes from the time of the metabolism of NA in isolated perfused lungs excised from BDL and sham-operated rats. The data is the mean ± S.E.M. of 4 to 5 animals per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3. A summary of the *in vitro* enzyme activities of rat lung homogenates prepared from sham-operated and BDL rats

	Activity (nmoles/mg protein/min)					
	N	MAO type A	N	MAO type B	N	COMT
Sham	18	2·6 ± 0·5	16	1·6 ± 0·4	8	0·013 ± 0·00
BDL	8	2·7 ± 0·5	8	1·7 ± 0·4	5	0·016 ± 0·00

Values shown are mean ± S.E.M.

respectively, BDL had no effect on the activities of these 2 enzymes. Table 3 summarizes the activities obtained in homogenates prepared from lungs from BDL and sham-operated rats. Although in BDL rats there appeared to be an apparent increase in COMT activity, this was not significant (Table 3).

Pulmonary perfusion pressure and the response to NA

The baseline pulmonary perfusion pressure in the BDL rats was 14.5 ± 1.7 mmHg and this was not significantly different to the perfusion pressure seen in sham-operated rats (14.8 ± 2.0 mmHg). Moreover, there were no differences in the dose–response curves to NA between the sham-operated and BDL rats (Fig. 2).

DISCUSSION

The pulmonary metabolism of NA in the rat is comprised of an active uptake process into the pulmonary endothelial cell followed by intracellular enzyme catabolism by MAO and COMT [10]. Utilizing an isolated perfused rat lung in which the integrity of the pulmonary endothelial cell membrane is intact, we have shown that the metabolism of NA is increased in lungs removed from BDL rats. Moreover, this increase was almost identical to the increase in the rate of uptake of NA into the lungs of the BDL rats. In a second series of experiments utilizing lung homogenates where the cell membrane of pulmonary endothelial cell is no longer intact and the catabolic enzymes are in direct contact with the specific substrates, we were unable to demonstrate any differences in the activities of MAO type A and B and COMT, between sham-operated and BDL rat lung homogenates. This finding is indicative that the change in the metabolism of NA by the lung is due to a change in the rate of uptake of NA and not with the activities of its catabolic enzymes.

The change in uptake of NA could not be related to the depth of jaundice or serum cholesterol concentrations. We did not measure serum bile salt concentrations in these animals and it is possible that these changes are correlated with increases in their concentration. Furthermore, these changes in NA metabolism and uptake occurred independent of any changes in pulmonary perfusion pressure or pressor responses to NA.

In a second series of experiments, we have demonstrated that the BDL-induced increase in the pulmonary metabolism of NA is time dependent, and occurred independently of the changes in the blood tests for liver function.

Although serum albumin levels fell in BDL rats from 3 to 6 days postoperatively, the increasingly significant differences in pulmonary metabolism of amines in this period is not reflected in differences in the albumin levels between the two groups. Furthermore, in view of the catabolic state of the BDL rats, it is difficult to draw any conclusions as to the role of hepatic synthetic function in the pathogenesis of the pulmonary metabolic abnormality.

Although we observed changes in blood tests for liver function in the sham-operated rats, we presume that they were due to manipulation of the bile duct as the values were not significantly different to our previously published control values [20].

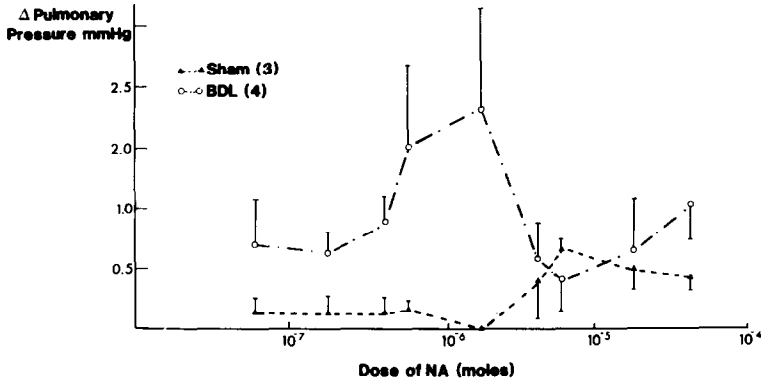

Fig. 2. The pressor response in 3-day sham-operated and BDL rats of the pulmonary vasculature to NA: ordinate, changes in pressor response in mmHg; abscissa, dose of NA. The numbers in brackets refer to sample size. Data is shown as mean ± S.E.M.

Since the metabolism of NA is greater at 6 days than at 3 days and the jaundice is less, what are the causes for these changes in metabolism? In this regard various alternatives exist. Firstly, the effects observed are due to other humoral factors that are elevated in obstructive jaundice that have not been measured in this study such as bile salts and endotoxin. Secondly, the uptake process may merely be responding to progressive increases in concentrations of circulating NA. The plasma NA concentrations in patients with obstructive jaundice is not known although Saito [2] and Geoffroy *et al.* [21] have demonstrated that they are elevated in animals whose bile ducts have been ligated. Despite the elevated levels of circulating NA seen in the BDL animals, the concentrations are still below the K_m of NA uptake in the lung. Thus, one of the ways in which BDL induces increases in pulmonary NA metabolism may be the induction of new uptake sites. At present, we do not know whether this is an effect on the V_{max} or K_m of NA uptake and this is currently being examined. Thirdly, sepsis is more common in surgical obstructive jaundice [22], but the effect of sepsis on the uptake processes or pulmonary metabolism is unknown. However, none of the blood cultures taken from jaundiced animals were positive.

The change in the metabolic capability of the lungs in obstructive jaundice provides a possible explanation for the loss of response to intravenously administered amines which would have to pass through the pulmonary circulation to reach the target site. Assuming that no pulmonary arteriovenous shunts exist, the observed response will be dependent not only upon the type of amine and its concentration, but also the duration of the obstruction. Such an effect can be readily seen in the studies of Finberg *et al.* [23] and Bomzon *et al.* [24] in conscious BDL dogs. These investigators demonstrated that there is a blunted pressor response following intravenous infusions of NA whereas the pressor response following intra-arterial infusion was unaffected by bile duct ligation. This finding indicates that the blunted pressor response following intravenous NA infusions may be due to increased pulmonary NA metabolism resulting in a reduced NA concentration reaching the heart. This explanation may be relevant to the discrepancy in vascular responses to amines with various types of liver disease such as fulminant hepatic failure [25] and cirrhosis. However, altered pulmonary amine metabolism cannot completely explain loss of pressor response in obstructive jaundice since it has recently been demonstrated that there is an impaired contractile response of isolated vascular tissue removed from bile duct ligated rats [26].

In conclusion, using the bile duct ligated rat as a model of obstructive jaundice, a disease associated with some cardiovascular instability, we have been able to demonstrate a change in the pulmonary metabolism of NA. On the basis of these results, it is tempting to speculate that if such changes in NA metabolism exist in other tissues that the vascular instability found in patients with liver disease is related to changes in peripheral NA metabolism.

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