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Ethanol Preference, Metabolism, Blood Pressure, and Conditioned Taste Aversion in Experimental Cholestasis

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LANE, J. R., E. M. STARBUCK AND D. A FITTS. *Ethanol preference, metabolism, blood pressure, and conditioned taste aversion in experimental cholestasis.* PHARMACOL BIOCHEM BEHAV **57**(4) 755–766, 1997.—The effect of a ligation of the common bile duct (BDL) on the chronic free-selection intake of ethanol was investigated. Rats were given a choice between water and a solution of either 6% (v/v) ethanol, 0.06% (w/v) sodium saccharin, or a mixture of both ethanol and saccharin. In different experiments, solutions were first presented either 3 weeks before surgery, about the time of surgery, or 2 weeks after surgery. Reductions in ethanol or saccharin intake were observed in BDL rats whenever the solutions were first presented either 3 weeks before or shortly after the surgery. No differences attributable to BDL were seen when ethanol solutions were first presented 2 weeks after surgery. The contingent nature of the effect suggests that the reduction results from a conditioned taste aversion rather than from differences in ethanol metabolism, sensitivity, or neurohormones such as angiotensin. The findings urge caution in the monitoring of the dietary habits of patients with a rapidly developing biliary obstruction. © 1997 Elsevier Science Inc.

Bile duct ligation Heart rate Saccharin preference Water intake

CIRRHOSIS of the liver is one of the leading causes of death in the Western world with over 90% of cirrhotic patients showing a pattern of unremitting daily alcohol consumption (20). The hepatic disease itself, regardless of its etiology, produces a variety of changes, including alterations in the cardiovascular and endocrine systems, that could affect the ingestion of alcohol. Among these many changes are: (A) chronic vasodilation due in part to portal hypertension; (B) high plasma monoamines and renin secretion; (C) hyperammonemia with a build up of the inhibitory neurotransmitter gamma aminobutryric acid (GABA); and (D) possible changes in ethanol metabolism or sensitivity (4,5,16,21). Numerous studies have explored the relationship between cirrhosis and the pattern and quantity of alcohol consumption in cirrhotic patients, but the extent to which the liver disease affects the ingestion of alcohol is unknown.

The aim of the present study was to explore the effects of cholestasis and cirrhosis on the chronic free selection of alcohol using a reliable and efficient animal model of cirrhosis. The most widely used animal models of cirrhosis include carbon tetrachloride inhalation, bile duct ligation (BDL), and chronic high dose ethanol ingestion usually in a liquid diet (5,18). We chose the BDL model because it is simple and reliable, it does not expose the rats to any organic solvents, and because it is a good model of human obstructive jaundice which occasionally occurs with pancreatitis or chronic alcohol ingestion (7,14). Importantly, the BDL model allowed us to determine the effects of cholestasis and cirrhosis on ethanol ingestion in rats that either did or did not have any substantial prior exposure to organic solvents such as ethanol.

In the first experiment, BDL and sham-ligated rats were given substantial exposure to the taste of an ethanol solution or a control saccharin solution before the surgical manipulation. In the second experiment, rats received either a BDL or sham ligation at least 2 weeks before they were first exposed to ethanol. In the third experiment, we optimized the association between an arbitrary flavor, saccharin, and a BDL surgery to determine the strength of a conditioned aversion to a highly palatable solution under a more commonly used protocol and without the postingestive effects of ethanol. Finally, we measured the rate of elimination of ethanol and the blood pressure response to ethanol in chronic BDL and sham-ligated rats. The

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results demonstrate that ethanol ingestion is reduced when the flavor is presented before, but not after, a BDL surgery. This suggests that the reduction in ethanol intake is mediated by a conditioned taste aversion and not by a reduction in ethanol metabolism, the renin-angiotensin system, or other unconditioned endocrine or physiological events.

GENERAL METHOD

Subjects

Subjects were male Long–Evans rats weighing between 200 and 500 g. They were housed individually in hanging wire mesh cages with Teklad laboratory chow and tap water continuously available except during experimentation. Room temperature was held at 23°C and lights were on 12 h per day. Sample sizes are given in the individual experiments.

Fluid and Food Intake

The fluids used in preference studies were either a 6% (v/v in tap water) ethanol solution, a saccharin solution (0.06% w/v sodium saccharin in tap water), or an ethanol-saccharin solution (sweetened ethanol) consisting of both 0.06% (w/v) sodium saccharin and 6% (v/v) ethanol. Solutions were always given in a two-bottle choice with tap water as the alternative except where noted. Fluid intakes were measured using 100 or 250 ml graduated cylinders fitted with stainless steel drinking spouts. All intakes were recorded to the nearest milliliter. The positions of the drinking tubes were alternated daily to control for position preferences.

In some experiments food intake was measured using the following procedure. At the beginning of the dark phase of the day:night cycle, food hoppers were filled with food, weighed, and placed into the cages. The following day the food hopper and crumbs collected from under the cage were weighed. Total food intake was calculated by subtracting this weight from the weight of the food hopper the previous day.

Surgeries

Some rats recieved either a BDL or a sham-ligation surgery under halothane anesthesia. BDL surgery was completed by ligating the common bile duct with 4–0 silk suture just above the duodenum anterior to the pancreas and posterior to the hilum of the liver, thereby eliminating the flow of bile from the liver into the duodenum. Another tie was made approximately 2 mm anterior to the first and the bile duct was then cut between the two ligatures to prevent the possibility of recannulation. A sham ligation surgery consisted of locating the bile duct, manipulating it, and replacing it. Animals were given topical Betadine as antiseptic and 0.2 ml Gentamicin IM to control for post surgical infection. Bupivicaine was used as a local anesthetic. All surgeries were performed using aseptic techniques.

Polyethylene catheters were inserted into the left femoral artery and vein of some rats with BDL or sham ligations under halothane anesthesia for later measurement of blood pressure and blood ethanol elimination. Catheters were constructed of PE-10 tubing heat welded to a longer piece of PE-50 tubing; the latter tubing was tunneled subcutaneously to an exit wound between the scapulae. The catheters were filled with 100 u/ml heparin in sterile isotonic saline and obturated until the time of the experiment 2–3 days later.

Blood Collection and Assays

At the end of some experiments, rats were weighed and then rapidly anesthetized with halothane, and a 10 ml sample of blood was drawn by cardiac puncture into syringes with heparinized needles. Hematocrit was measured by the microhematocrit method, and, when relevant, 0.05 ml of blood was pipetted into 0.45 ml of trichloroacetic acid and frozen for later determination of blood ethanol concentration using a spectrophotometric kit from Sigma Chemical (St. Louis, Mo., kit #333-UV). The remaining blood was centrifuged to obtain plasma. Plasma osmolality and protein were immediately determined by freezing point depression and refractometry methods, respectively. The residual plasma was frozen for later determination of plasma sodium and potassium concentrations by flame photometry and total plasma bilirubin concentration by spectrophotometry (Sigma kit #552).

Necropsy

Following blood collection, rats were given an overdose of pentobarbital sodium by intracardiac injection. Ascites fluid accumulation and degree of bloating of the bile duct stump were noted qualitatively. In addition, the liver, kidneys, and heart were dissected, blotted dry, and weighed.

In each experiment either urine or plasma was analyzed to verify cholestasis. If blood was collected, plasma bilirubin concentration was analyzed quantitatively except as noted in the tables. In other experiments urine was collected by free catch in metabolic cages at least 3 weeks following BDL and qualitatively screened for bilirubin using Ames Icotest Reagent Tablets. These procedures verified the presence of cholestasis and assured that bile duct recannulation had not occurred in any of the animals.

General Procedure

Rats were randomly assigned to a BDL or sham-ligation treatment except where noted. All rats in the preference experiments received either an ethanol, sweetened ethanol, or saccharin solution in choice with tap water before or after the surgical or experimental treatment. In one experiment, rats were given only water or sweetened ethanol to drink without a choice. Blood collection and necropsies were performed on the last day of intakes in each of the experiments unless stated otherwise. The intakes were averaged over 3 or 4 days before analysis.

Experiment 1: Prior Access to Ethanol or Saccharin

The following experiment was designed to examine the effects of a BDL or sham ligation on the free selection intake of an ethanol, saccharin, or a sweetened ethanol solution, by rats that have had substantial prior experience with the target flavor. Each solution was administered to different groups in a two-bottle choice with tap water as the alternative. The groups given 6% ethanol and water included 10 BDL and 9 sham-ligated rats. These rats received access to ethanol for 21 days prior to surgery and for 36 days after surgery. Food intake was measured for 12 h immediately before necropsy. The groups given 0.06% saccharin without ethanol included 12 BDL and 7 sham-ligated rats. These rats received access to saccharin for 22 days prior to surgery and for 30 days after surgery. Food intake was not measured. The groups given a mixture of 6% ethanol and 0.06% saccharin included 12 BDL and 11 sham-ligated rats. These rats received access to the

sweetened ethanol solution for 18 days prior to surgery and for 29 days after surgery. Food intake was not measured.

Experiment 2: Delayed Access to Ethanol or Sweetened Ethanol

The following experiment was designed to examine the effects of a BDL or sham ligation on the free selection intake of an ethanol solution or a sweetened ethanol solution by rats that have had no experience with the target flavor before surgery. The groups given 6% ethanol included 12 BDL and 11 sham-ligated rats. These rats received access to the ethanol solution for 28 days beginning 15 days after surgery. Food intake was not measured. The groups given a mixture of 6% ethanol and 0.06% saccharin included 8 BDL and 6 sham-ligated rats. These rats received access to the sweetened ethanol solution for 29 days beginning 15 days after surgery. Food intake was measured for 1 day 25 days after the surgery (4 days before necropsy).

Experiment 3: Forced-Choice Access to Sweetened Ethanol or Water

This experiment was designed to test the effects of BDL or sham ligation on consumption of a sweetened ethanol solution or water in a one-bottle test. The groups receiving sweetened ethanol included 8 BDL and 8 sham-ligated rats. The groups receiving only water included 7 BDL and 7 sham-ligated rats. Three days after surgery, the water-only groups received tap water as usual and the sweetened-ethanol group received a choice between water and sweetened ethanol for 3 days of adaptation. On the sixth day after surgery, the water-only rats continued on the same regimen and the sweetened-ethanol group received only an ethanol-saccharin solution for 21 additional days. Food intake was not measured.

Experiment 4: Conditioned Taste Aversion with Saccharin

This experiment was designed to optimize the conditions for producing an association between a BDL surgery and an arbitrary, novel flavor, such as saccharin. In a previous experiment, rats having prior experience with saccharin did not develop a potent aversion for the flavor. If the BDL surgery does produce discomfort that can become associated with a novel flavor, then a close temporal pairing of the novel flavor with the BDL surgery should cause the rats to develop an aversion even for the highly palatable saccharin solution.

Fourteen rats were weighed and deprived of food and water overnight. The next day, the rats were given a single bottle of 0.06% saccharin solution, and intakes were recorded for 2 h. The onset of each rat's drinking test was timed so that the rat could receive either a BDL or sham-ligation surgery exactly 2 h after the presentation of saccharin ($n = 7$ each). This group is referred to as the paired group.

Ten other rats were treated identically except that the saccharin solution was removed after 2 h, food and water were returned ad lib, and the BDL or sham surgery was conducted 48 h after exposure to the novel saccharin flavor instead of immediately ($n = 5$ each). This group is referred to as the nonpaired group.

For comparison with a more traditional procedure, two additional groups of rats were exposed to the saccharin solution paired 2 h later with an IP injection of 20 ml/kg of either isotonic lithium chloride or isotonic sodium chloride ($n = 3$ each).

After the surgical or injection treatments, the rats were offered a choice between water and saccharin solution, and their intakes were recorded daily for 27 days. Urine was collected from all rats receiving a surgical treatment in order to verify cholestasis. Blood collection and necropsies were not performed on rats in this experiment.

Experiment 5: Blood Ethanol Elimination and Blood Pressure

This experiment was designed to determine if the large differences in ethanol intake observed in previous experiments could be explained by changes in the elimination of ethanol after BDL surgery. Furthermore, blood pressure was measured during both baseline and intoxicated states to determine if the BDL surgery produced hypotension that has previously been associated with high renin and angiotensin levels in cirrhotic rats and to determine the effects of ethanol. Catheters were implanted into both the left femoral artery and vein of rats 4 weeks after BDL ($n = 10$) or sham ligation ($n = 8$). Two or 3 days later, the rats were transferred to cylindrical Plexiglas cages for ethanol infusion, blood sampling, and blood pressure measurement. The venous catheter was connected via PE-50 tubing to a 20-ml syringe on a Harvard infusion pump. The arterial catheter was connected to a Cobe blood pressure transducer, and the rat was allowed to rest undisturbed for 15 min before the experiment. Because of equipment limitations, blood pressure was recorded on only 8 of the BDL rats and 6 of the sham-ligated rats. The amplified pressure signal was digitized at 100 Hz with a Labmaster AD board and stored on disk in an IBM AT computer. The pressure wave was later analyzed for mean arterial pressure and heart rate. Blood pressure was recorded for at least 30 min, and 10 min of stable pressure data at the end of this period were used as a baseline value. The infusion pumps were then turned on, and each rat received 1.5 g/kg ethanol at a rate of 0.3 ml/min for 15 min. The concentration of ethanol, which varied according to the body weight of each rat, ranged from 12 to 21% w/v. The pumps were turned off after 15 min of infusion, and 0.1-ml blood samples were drawn through the arterial catheter at 20, 75, and 135 min after the beginning of the infusion (or 5, 60 and 120 min after the end of the infusion). From each sample, 0.05 ml of blood was immediately pipetted into 0.45 ml trichloroacetic acid and frozen for later determination of ethanol concentration. At the end of the experiment, an additional blood sample was drawn for plasma bilirubin determinations. Blood pressure and heart rate were averaged for the following 5 periods: (A) the 10 min baseline period; (B) the 15 min infusion period; (C) the next 4 min after the infusion before the first blood sample; (D) the next 52 min before the second blood sample; and (E) the next 60 min before the final blood sample. The pressure transducers were switched offline with a 3-way stopcock for about 1–2 min while blood samples were being drawn. Necropsies were not performed on the rats in this experiment.

Experimental Design and Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) appropriate to the individual designs. Water intake and ethanol, sweetened ethanol and saccharin intakes were analyzed using a mixed model ANOVA with surgical condition as the between subjects factor and 3- or 4-day blocks as the within subjects factor unless specified otherwise. Planned comparisons were made using Fisher's least significant difference test

or the Bonferroni test if the F was not significant. A probability of less than 0.05 was required for significance.

RESULTS

General Mortality

Our study was not designed to measure the effects of ethanol or BDL surgery on mortality in rats, but we did observe rapid degeneration in 14 BDL rats leading to death or euthanasia in the five experiments. None of the 80 sham-ligated rats died. The total numbers of successful BDL preparations were 47 for the chronic ethanol treatments and 44 for the non-ethanol treatments and the acute ethanol-infusion treatment. Of the 14 BDL mortalities, 8 received ethanol or sweetened ethanol as a chronic treatment, and 6 received no ethanol. Although these death rates were similar, there were several coincidences in which the highest ethanol-consuming BDL rats happened to be the ones that died in the experiment. For this reason, the individual data for these mortalities are given in the results sections for each experiment.

Experiment 1: Prior Access to Ethanol or Saccharin

Of the rats receiving 6% ethanol in choice with water prior to surgery, 1 BDL rat died 6 days before the scheduled blood collection and necropsy. Therefore, the fluid and preference data and all necropsy data are based on 9 BDL rats. Of the rats receiving 0.06% saccharin solution in choice with water prior to surgery, 3 BDL rats were eliminated from the analyses because of normal plasma bilirubin concentrations (5.7, 3.8, and 3.8 μMol/L), and 1 BDL rat was euthanized on the day before blood collection and necropsy. Of the rats receiving sweetened ethanol in choice with water, 1 BDL rat died 3 days after surgery, and 2 more BDL rats were euthanized 13 days after surgery. No sham-ligated rats died in this experiment. The necropsy data were combined with the results from the delayed access experiments and are presented below.

Unadulterated Ethanol

The drinking data for the groups receiving 6% ethanol in choice with water before surgery were averaged in 4-day blocks and are displayed in Fig. 1.

Before the surgery, rats showed a typical acclimation to ethanol: All rats increased their ethanol intake and decreased their water intake during the first 5 blocks prior to surgery. The rats' stable intakes during the last block were used as a presurgical baseline for a planned within-group comparison with combined postsurgical blocks. BDL rats significantly increased water intake during the postsurgical period, $F(1, 220) = 10.88, p < 0.001$, and ingested significantly less ethanol during the postsurgical period, $F(1, 220) = 4.01, p < 0.05$. Sham-ligated rats did not significantly alter water or ethanol intake during the postsurgical period. Comparisons between BDL and sham-ligated groups supported this finding, with BDL rats drinking less ethanol, $F(1, 220) = 48.01, p < 0.001$, and more water, $F(1, 220) = 27.05, p < 0.001$, than sham-ligated rats during the 9 blocks following the surgery. The groups did not differ significantly in ethanol intake before surgery.

The daily intakes of all rats were stable and well represented by the 4-day blocks presented in the figure except for the critical days at the time of surgery. The ethanol intake of the sham-ligated group was barely affected by the laparotomy, whereas the intakes of the BDL group showed an immediate

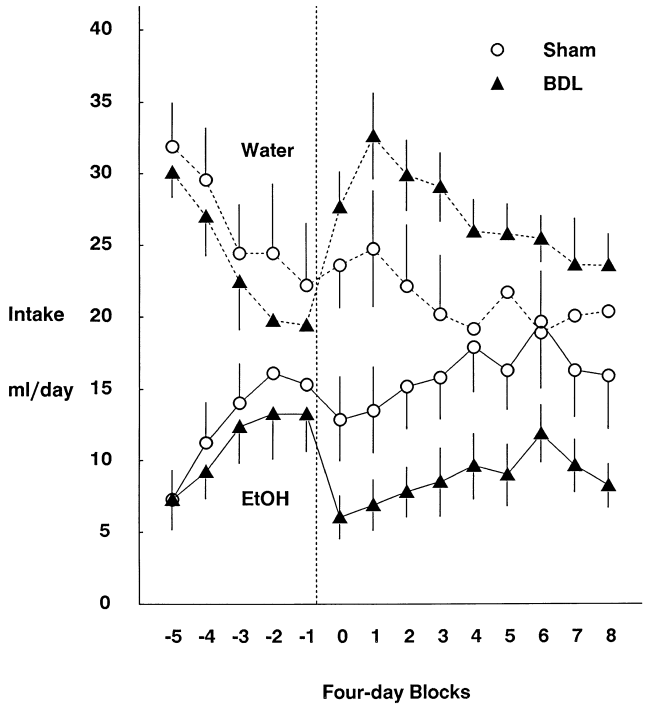

FIG. 1. Daily intakes of rats receiving 6% ethanol in choice with water for 21 days before and 36 days after BDL or sham-ligation surgery (vertical dashed line). Means and standard errors of 4-day blocks.

decline on day 1, a recovery almost to the presurgical level on day 2, and a more permanent decline on days 3 and following.

The one BDL rat that died 6 days before the end of the experiment consumed by far the most alcohol of any rat in the group both before and after the ligation operation and averaged 22 ml/day of ethanol solution on the 2 days before his death. The average of those 2 days was used as the rat's score on the penultimate block, and also on the last block in order to avoid eliminating the rat entirely from the analysis. One degree of freedom was removed from the error term of subsequent analyses to compensate for the replacement of the data point.

Food intake by the sham-ligated rats on the last day before necropsy ranged from 18–26 g (4.5–5.4 g/100 g body weight). All but 3 rats in the BDL group ate amounts within this range. Of the 3 poor eaters, 1 rat was completely aphagic by the day of necropsy. This rat, which drank 11 ml water and 7 ml ethanol on that day, had the second highest average postsurgical ethanol intake among the BDL rats in the experiment (15 ml/day) despite having the lowest body weight of the group (337 g at necropsy). Both this rat and the rat that died had also been relatively high drinkers before the surgery. Thus, habitually high ethanol intake by certain BDL rats seemed to be detrimental to their general health.

Unadulterated Saccharin

The drinking data for the groups receiving 0.06% saccharin in choice with water prior to surgery were averaged in 4-day blocks and are displayed in Fig. 2. The last block of days before surgery was used as a baseline for within-subjects comparisions of water and saccharin intakes after surgery. The

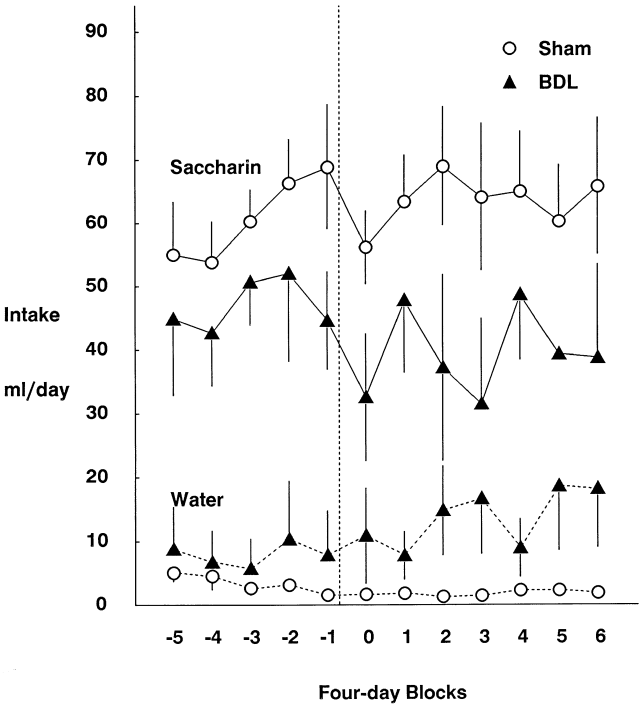

FIG. 2. Daily intakes of rats receiving 0.06% saccharin in choice with water for 22 days before and 30 days after BDL or sham-ligation surgery (vertical dashed line). Means and standard errors of 4-day blocks.

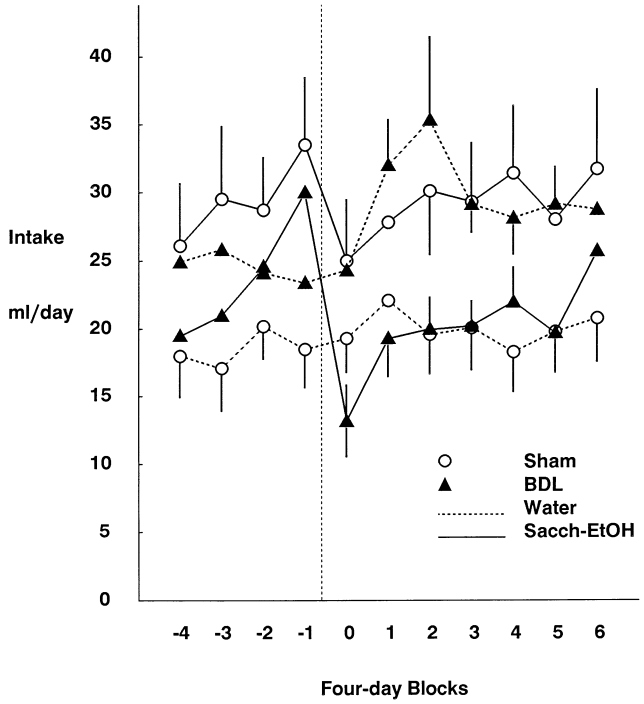

FIG. 3. Daily intakes of rats receiving a mixture of 6% ethanol and 0.06% saccharin in choice with water for 18 days before and 29 days after BDL or sham-ligation surgery (vertical dashed line). Means and standard errors of 4-day blocks.

small increase from baseline water intake after BDL surgery was not statistically significant, but the saccharin intake by BDL rats was significantly reduced from baseline after the surgery, $F(1, 154) = 21.11$, $p < 0.001$. Sham ligated rats drank the same amounts of water and saccharin before and after surgery. Between-subjects comparisons revealed that BDL rats drank significantly more water, $F(1, 154) = 23.48$, $p < 0.001$, and less saccharin, $F(1, 154) = 33.08$, $p < 0.001$, than sham-ligated rats after surgery.

Ethanol and Saccharin Combined

The drinking data for the groups receiving a mixture of 6% ethanol and 0.06% saccharin in choice with water prior to surgery were averaged in 4-day blocks and are displayed in Fig. 3.

The BDL rats that received a sweetened ethanol solution persistently decreased solution intake, $F(1, 180) = 10.52$, $p < 0.001$, and increased water intake, $F(1, 180) = 3.87$, $p < 0.05$, after the surgery compared with their own baseline intakes. The BDL rats also had significantly reduced solution intakes and enhanced water intakes after surgery compared with the sham-ligated control rats. However, this effect was confounded by the fact that the BDL group also had significantly lower solution intakes and higher water intakes than the sham-ligated group before the surgery. The three rats that died and were eliminated from the analyses consumed on average 27, 38, and 41 ml of sweetened ethanol solution per day during the entire baseline period, and these were among the highest intakes in the group. Their elimination thus resulted in a significant reduction in the group's mean intake. Further analysis of the data of the surviving rats revealed that the decrease of the solution intake from baseline was greater in the BDL than in the sham-ligated group, $F(1, 180) = 9.88$, $p < 0.005$, and that the increase of the water intake from baseline was greater in the BDL than in the sham-ligated group, $F(1, 180) = 10.55$, $p < 0.005$. Thus, the decrease in solution intake after ligation was not merely a function of a lower initial preference in these animals.

Experiment 2: Delayed Access to Ethanol or Sweetened Ethanol

Of the rats receiving ethanol in choice with water 15 days after surgery, 2 BDL rats were eliminated from the analysis because of normal total plasma bilirubin concentrations (3.4 and 5.1 μMol/L), and a third rat with massive ascites accumulation was euthanized 2 days prior to blood collection and necropsy. Of the rats receiving a mixture of 6% ethanol and 0.06% saccharin in choice with water 15 days after surgery, 1 BDL rat was eliminated from the analysis because of normal total plasma bilirubin concentrations (7.5 μMol/L). No BDL rats receiving sweetened ethanol died, and no sham-ligated rats died during either phase of the experiment.

Unadulterated Ethanol

The ethanol and water intakes for the groups receiving 6% ethanol in choice with water 15 days after surgery were averaged in 4-day blocks and are shown in Fig. 4. BDL rats drank more water (38.7 ± 3.2) than sham-ligated rats (31.5 ± 1.4) over the duration of the experiment, main effect of surgery, $F(1, 16) = 5.79$, $p < 0.05$. Ethanol intake was not different between BDL and sham-ligated rats. The rat that

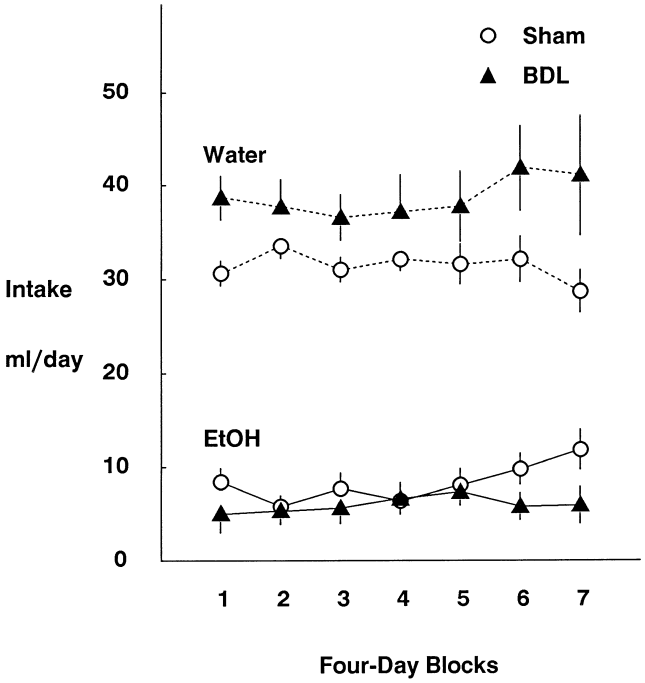

FIG. 4. Daily intakes of rats receiving 6% ethanol in choice with water for 28 days beginning 15 days after BDL or sham-ligation surgery. Means and standard errors of 4-day blocks.

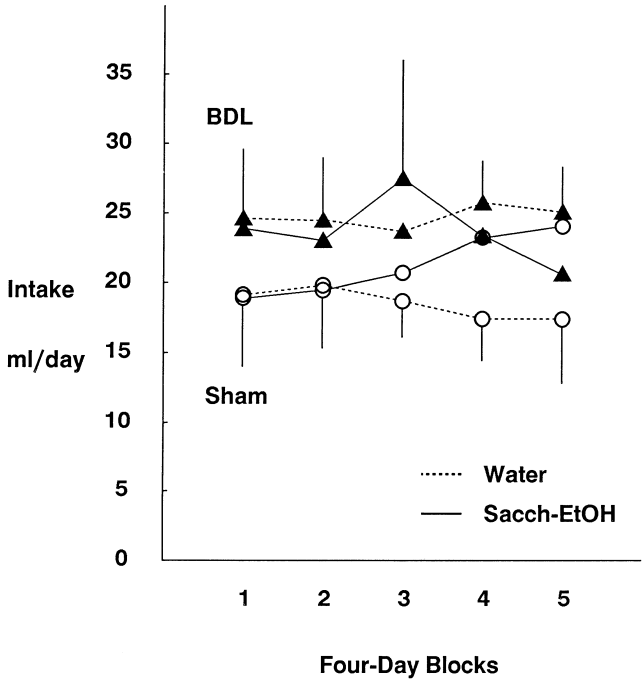

FIG. 5. Daily intakes of rats receiving a mixture of 6% ethanol and 0.06% saccharin in choice with water for 29 days beginning 15 days after BDL or sham-ligation surgery. Means and standard errors of 4-day blocks.

died 2 days before the end of the experiment was included in the analysis, with the mean of the last 4-day block for that animal being represented by the average intake for the existing 2 days in the block. The ethanol intake by this animal was near the mean of its group during the first week of exposure, and lower than the mean thereafter. The general conclusion would not have been altered by the exclusion of this rat.

Ethanol and Saccharin Combined

The fluid intake data were averaged in 4-day blocks and are shown in Fig. 5. There were no significant differences in either water intake or sweetened ethanol intake among the groups.

Food intake by the sham-ligated rats 25 days after the surgical manipulation (10 days access to sweetened ethanol) ranged from 21–28 g (6.3–7.4 g/100 g body weight). The food intake of 3 of the 7 BDL rats did not fall within this range (16–20 g or 5.4–6.0 g/100 g body weight). These 3 BDL rats drank the largest amounts of the sweetened ethanol solution, and this apparently constituted much of their daily caloric intake.

Blood and Necropsy Data

The blood and necropsy data from Experiments 1 and 2 for the two-bottle prior access and delayed access to ethanol experiments are presented in Table 1 for the unadulterated ethanol experiments and in Table 2 for the sweetened ethanol experiments. The data were analyzed by ANOVA using experiments (prior access versus delayed access) and surgical condition as between-subjects factors. Data were included only for those animals that survived until the day of necropsy.

Blood alcohol levels in these experiments were negligible as has frequently been observed in studies of free-choice alcohol intake by rodents when blood samples were collected during the light part of the daily cycle (15). The highest observed blood alcohol concentration was 20 mg/dl, and most were merely trace amounts. As a consequence, no effort has been made to correct the plasma osmolalities for the presence of alcohol (10).

BDL rats gained significantly less weight than sham-ligated rats in all experiments. At the time of necropsy, all groups of BDL rats had significantly heavier livers and kidneys than the sham-ligated control rats. This corroborates the conclusion drawn from plasma and urine bilirubin analyses and direct observation of the dissected bile duct that the BDL procedure was effective in producing cholestasis and cirrhosis. Relative heart weights were also heavier in BDL groups in the experiments where heart weight was measured.

The plasmas of the BDL groups were typically hypoosmotic compared with the plasmas of sham-ligated groups. The only two-bottle preference experiment where this was not true was the prior access to saccharin experiment without ethanol (not shown). In that experiment, plasma osmolality of the BDL group (303.8 $\pm$ 2.3 mosmol/kg) was similar to BDL groups in other experiments, but the osmolalities of the sham-ligated rats (300.7 $\pm$ 2.7 mosmol/kg) appeared somewhat lower than those of the sham-ligated groups in other experiments. This suggests that the high fluid turnover in these animals may have slightly suppressed osmolality in the control rats.

Plasma sodium and potassium concentrations did not differ significantly between BDL and sham-ligated rats in any two-bottle preference experiment. Plasma protein concentrations were similar between BDL and sham-ligated groups in all two-bottle alcohol experiments, but in the saccharin preference

TABLE 1

BLOOD AND NECROPSY DATA 36 OR 43 DAYS AFTER A BILE DUCT LIGATION (BDL) OR A SHAM LIGATION IN RATS FROM EXPERIMENTS 1 AND 2 HAVING FREE SELECTION ACCESS TO 6% ETHANOL BEGINNING EITHER 21 DAYS PRIOR TO (PRIOR ACCESS) OR 28 DAYS AFTER SURGERY (DELAYED ACCESS)

	Prior Access		Delayed Access	
Variable	BDL	Sham	BDL	Sham
Body weight data				
n	9	9	6	11
Preligation BW (g)	411 ± 8	421 ± 11	395 ± 13	392 ± 13
Necropsy BW (g)	428 ± 17*	478 ± 14	438 ± 22*	473 ± 17
BW (%Δ)	4 ± 2*	13 ± 1	12 ± 3*	21 ± 2
Blood data				
Hct (%)	49.2 ± 1.5*	46.3 ± 0.6	50.5 ± 1.6*	47 ± 0.5
PP (g/dl)	5.8 ± 0.3	6.1 ± 0.1	6.1 ± 0.2	6.0 ± 0.2
P_{osm} (mOsm/kg)	297 ± 2*	303 ± 1	297 ± 2*	305 ± 1
P_{Na} (mmol/L)	126 ± 3	126 ± 4	127 ± 1	128 ± 0
P_K (mmol/L)	5.1 ± 0.2	5.4 ± 0.3	5.3 ± 0.2	5.4 ± 0.2
P_{Bili} (μMol/L)	NA	NA	108 ± 19	NA
Organ weight				
Liver (g)	25.4 ± 2.1*	19.1 ± 0.8	30.1 ± 3.0*	19.0 ± 1.0
(g/100 g)	5.9 ± 0.5*	4.0 ± 0.1	6.8 ± 0.3*	4.0 ± 0.1
Kidneys (g)	3.8 ± 0.2*	3.4 ± 0.1	4.5 ± 0.3*‡	3.3 ± 0.1
(g/100 g)	0.9 ± 0.02*	0.7 ± 0.03	1.0 ± 0.02*‡	0.7 ± 0.02
Heart (g)	NA	NA	1.9 ± 0.2*	1.4 ± 0.2
(g/100 g)	NA	NA	0.4 ± 0.03*	0.3 ± 0.02

Mean ± SE. Abbreviations: Hct, hematocrit; PP, plasma protein concentration; P_{osm}, plasma osmolality; P_{Na}, P_K, P_{Bili}, plasma sodium, potassium, and bilirubin concentrations. Three rats in the BDL group had ascites fluid accumulation.

*Main effect of surgical condition, $p < 0.05$; ‡interaction of surgical condition with experiment, $p < 0.05$; NA, not available.

experiment without ethanol the plasma protein concentrations were higher in the BDL group (7.4 ± 0.1 mg/dL) than in the sham-ligated group (6.2 ± 0.1 mg/dL).

Hematocrits did not vary consistently between BDL and sham-ligated rats in the preference experiments. Hematocrit was significantly elevated in BDL rats compared with sham-ligated rats in the prior access and delayed access experiments with unadulterated ethanol (Table 1). In the experiments with sweetened ethanol the hematocrits either did not differ or were reduced in BDL rats (Table 2), possibly because of the elevated fluid consumption of these rats. Hematocrits were also similar between groups of BDL rats (48.4 ± 2.1%) and sham-ligated rats (48.9 ± 0.7%) receiving saccharin without ethanol, suggesting that the volume of fluid consumed as well as the surgical condition and drug treatment may influence the hematocrit in these experiments.

In all of the experiments, the livers of BDL rats were mottled with green or yellow, hard, and fibrotic. The kidneys of BDL rats were dark brown or black instead of their normal reddish brown. By gross examination, the hearts of BDL rats appeared normal in color and texture. The stump of the ligated and cut bile duct was often black and engorged with bile (about 2 cm diameter). In the other BDL rats, the bile duct was smaller and yellowish but was clearly ligated and cut, and these animals tested positive for hyperbilirubinuria or hyperbilirubinemia. Abnormal bile ducts were not observed in any sham-ligated rats. Ascites accumulation was present in 3 out of 6 BDL rats in the delayed access to ethanol experiment and in 3 out of 7 BDL rats in the delayed access to sweetened ethanol experiment. The presence of ascites in these rats is not unusual given the long delay (43 and 44 days) after the ligation. Steatosis, sometimes a symptom of cirrhosis, is characterized by a pale and often yellow liver, while cirrhosis in general is characterized by a small, normal or large liver which is firm because of the presence of fibrous septa (12).

Experiment 3: Forced-Choice Access to Sweetened Ethanol or Water

Three days after BDL or sham-ligation surgery, rats in this experiment were given either water alone or water in choice with sweetened ethanol to drink. Ligation surgery did not affect water intake by the water-only animals, but the BDL rats with a choice drank significantly less alcohol ($t(14) = 2.34$, $p = 0.035$) than the sham-ligated rats during this 3-day adaptation period. The intake of sweetened ethanol averaged 10 ± 3 ml/day in the BDL group and 24 ± 5 ml/day in the sham-ligated group. Concurrent water intakes by these groups were 27 ± 2 and 18 ± 3 ml/day, respectively.

For the next 21 days, the rats that had received water to drink continued drinking only water, and the rats that had received a choice between water and sweetened ethanol received only the sweetened ethanol to drink. Again, the ligation surgery did not significantly affect intake in the water-only groups. Sweetened ethanol intakes likewise did not differ between the BDL and sham-ligated groups as the BDL rats increased their ethanol intake from the period of choice to the period of no choice. Rats in both surgical conditions drank about 8 ml more fluid if they had water than if they had sweetened ethanol to drink, $F(1, 23) = 6.25$, $p = 0.02$.

TABLE 2

BLOOD AND NECROPSY DATA 29 OR 44 DAYS AFTER A BILE DUCT LIGATION (BDL) OR A SHAM LIGATION IN RATS FROM EXPERIMENTS 1 AND 2 HAVING FREE SELECTION ACCESS TO SWEETENED ETHANOL BEGINNING EITHER 18 DAYS PRIOR TO (PRIOR ACCESS) OR 29 DAYS AFTER SURGERY (DELAYED ACCESS)

	Prior Access		Delayed Access	
Variable	BDL	Sham	BDL	Sham
Body weight data				
n	9	11	7	6
Preligation BW (g)	340 ± 17	356 ± 18	229 ± 7	230 ± 7
Necropsy BW (g)	414 ± 15*	486 ± 17	354 ± 16*	394 ± 15
BW (%Δ)	24 ± 6	38 ± 5	55 ± 8	73 ± 9
Blood data				
Hct (%)	44.8 ± 1.8	48.3 ± 0.6	47.6 ± 0.8	47.9 ± 0.5
PP (g/dl)	6.6 ± 0.4	6.4 ± 0.1	6.0 ± 0.2	5.9 ± 0.1
P_{osm} (mOsm/kg)	302.7 ± 1.5*	307.9 ± 0.8	298.4 ± 1.4*	303.5 ± 1.4
P_{Na} (mmol/L)	132.7 ± 0.6	133.8 ± 0.4	131.4 ± 1.11	132.2 ± 0.5
P_K (mmol/L)	5.4 ± 0.2	5.4 ± 0.2	5.3 ± 0.3	5.5 ± 0.1
P_{Bili} (μMol/L)	NA	NA	157 ± 6	6 ± 1
Organ weight				
Liver (g)	29.3 ± 1.3*	20.0 ± 0.9	25.1 ± 2.1*	16.2 ± 0.4
(g/100 g)	7.1 ± 0.2*	4.1 ± 0.1	7.1 ± 0.4*	4.1 ± 0.1
Kidneys (g)	3.9 ± 0.2*	3.4 ± 0.1	3.7 ± 0.2*	3.0 ± 0.1
(g/100 g)	1.0 ± 0.04*	0.7 ± 0.02	1.2 ± 0.04*	0.8 ± 0.04
Heart (g)	1.4 ± 0.04	1.6 ± 0.1	1.6 ± 0.1‡	1.3 ± 0.1
(g/100 g)	0.4 ± 0.01*	0.3 ± 0.01	0.5 ± 0.03*‡	0.3 ± 0.01

Mean ± SE. Abbreviations: Hct, hematocrit; PP, plasma protein concentration; P_{osm}, plasma osmolality; P_{Na}, P_K, P_{Bili}, plasma sodium, potassium, and bilirubin concentrations.

*Main effect of surgical condition, $p < 0.05$; ‡interaction of surgical condition with experiment, $p < 0.05$.

Of the 3 BDL rats that died in the sweetened ethanol group, 1 showed a total avoidance of the solution during the choice phase, averaging 31 ml water and 1 ml ethanol during the 3 days. This animal was totally adipsic during the forced-choice phase of the experiment and was euthanized. The 2 other BDL rats showed a relative aversion to sweetened ethanol during the choice phase compared with the average consumption of sham-ligated animals, but they recovered their intakes in the forced-choice phase before dying 17 and 20 days after surgery. Two BDL rats in the water-only group were euthanized 7 and 8 days after ligation. No sham-ligated rats died.

As in the free-choice experiments, the blood alcohol levels of the rats in this experiment were negligible when sampled during the middle of the light part of the daily cycle. Necropsy data for this experiment are presented in Table 3. Hematocrit was significantly reduced in BDL rats, $F(1, 22) = 8.23$, $p = 0.009$. Plasma protein concentrations were significantly affected by surgical treatment, $F(1, 22) = 16.44$, $p = 0.001$, as well as by an interaction of surgery with the fluid treatment, $F(1, 22) = 4.32$, $p = 0.05$. The interaction was also significant for plasma sodium concentration, $F(1, 22) = 6.98$, $p < 0.05$. As in all experiments, the BDL rats in this experiment had significantly heavier livers (g, $p < 0.001$) and relatively heavier kidneys (g/100 g, $p < 0.003$) than sham-ligated rats.

Experiment 4: Conditioned Taste Aversion with Saccharin

Rats lost an average of 22 g following the 24 h period of food and water deprivation. At the onset of the drinking test, all rats immediately consumed the saccharin solution, drinking an average of 16.3 ml in 2 h. The paired and nonpaired groups drank similar amounts.

The chronic daily water and saccharin solution intakes for paired and nonpaired BDL and sham-ligated rats are presented in Fig. 6, averaged as 3-day blocks. In this design, surgical condition and pairing condition were the between-subjects factors and blocks was the within-subjects factor. Three nonpaired BDL rats that had normal urinary bilirubin were excluded from the analysis. Paired BDL rats consistently ingested less saccharin than paired sham-ligated rats throughout the duration of the experiment, whereas nonpaired BDL rats consumed amounts of saccharin solution comparable to nonpaired sham-ligated rats on blocks 1, 7, 8, and 9 but not on blocks 2–6, pairing-by-surgery-by-block interaction, $F(8, 136) = 2.69$, $p < 0.05$. In addition, paired BDL rats ingested significantly less saccharin than nonpaired BDL rats on blocks 4, 7, 8 and 9. Thus, some of the differences in saccharin ingestion between paired BDL versus nonpaired BDL rats can be attributed to the increased intake of saccharin in the nonpaired BDL group that occurred about 21 days after the surgery.

Inspection of the individual data showed that all paired BDL rats avoided the saccharin immediately after surgery, and only 2 of 7 paired BDL rats recovered their preference for saccharin during the next 27 days (days 8 and 13). The other 5 paired BDL rats drank an average of less than 2 ml/day of saccharin solution for the entire experiment. Conversely, all 5 nonpaired BDL rats had multiple days of drinking more than 10 ml of saccharin, usually toward the end of the 27-day test.

Water intake and saccharin intake for unoperated rats re-

TABLE 3

BLOOD AND NECROPSY DATA 24 DAYS AFTER A BILE DUCT LIGATION (BDL) OR A SHAM LIGATION IN RATS FROM EXPERIMENT 3 HAVING ACCESS TO WATER ALONE THROUGHOUT OR A CHOICE BETWEEN WATER AND SWEETENED ETHANOL FOR DAYS 3–6 AND A FORCED CHOICE ACCESS TO SWEETENED ETHANOL FOR DAYS 6–24 AFTER SURGERY

	Sweetened Ethanol		Water Only	
Variable	BDL	Sham	BDL	Sham
Body weight data				
n	8	8	7	7
Preligation BW (g)	324 ± 5	349 ± 6	342 ± 12	361 ± 10
Necropsy BW (g)	372 ± 14	410 ± 9	388 ± 11	417 ± 15
BW (%Δ)	9 ± 4	18 ± 1	14 ± 2	16 ± 2
Blood data				
Hct (%)	41.2 ± 1.8	46.0 ± 0.8	43.8 ± 0.9	44.9 ± 0.9
PP (g/dl)	6.6 ± 0.2	6.2 ± 0.2	7.1 ± 0.2	6.1 ± 0.1
P_{osm} (mOsm/kg)	305.4 ± 2.3	307.6 ± 1.0	308.0 ± 0.5	306.7 ± 0.9
P_{Na} (mmol/L)	131.2 ± 0.5	134.0 ± 0.8	133.5 ± 0.6	132.7 ± 0.8
P_K (mmol/L)	5.5 ± 0.3	5.5 ± 0.2	5.9 ± 0.2	5.2 ± 0.1
P_{Bili} (μMol/L)	103 ± 24	9 ± 1	93 ± 25	10 ± 1
Organ weight				
Liver (g)	24.3 ± 2.1*	17.2 ± 1.2	25.1 ± 2.3*	16.6 ± 0.9
(g/100 g)	6.6 ± 0.6*	4.2 ± 0.1	6.4 ± 0.5*	4.0 ± 0.1
Kidneys (g)	3.1 ± 0.2	2.9 ± 0.1	3.2 ± 0.2	3.0 ± 0.1
(g/100 g)	0.8 ± 0.05*	0.7 ± 0.02	0.8 ± 0.04*	0.7 ± 0.02
Heart (g)	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
(g/100 g)	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.02

Mean ± SE. Abbreviations: Hct, hematocrit; PP, plasma protein concentration; P_{osm}, plasma osmolality; P_{Na}, P_K, P_{Bili}, plasma sodium, potassium, and bilirubin concentrations.

*Main effect of surgical condition, $p < 0.05$; ‡interaction of surgical condition with experiment, $p < 0.05$.

ceiving either a lithium chloride (LiCl) or sodium chloride (NaCl) injection paired with saccharin ingestion were averaged as 3-day blocks (data not shown). LiCl-injected rats had higher water intakes and lower saccharin intakes than NaCl-injected rats on the first block. All 3 LiCl-injected rats drank less of the saccharin solution (range 0-1 ml) than the 3 NaCl-injected rats (range 20–60 ml) during the first 3-day block. Two LiCl-injected rats subsequently lost the aversion and drank copious amounts of saccharin solution for the remainder of the experiment. Rats injected with LiCl ingested more water (28.6 ± 5.3 ml/day) overall than rats injected with NaCl (8.4 ± 1.0 ml/day), main effect of injectate $F(1, 4) = 14.25$, $p < 0.05$.

Experiment 5: Blood Ethanol Elimination and Blood Pressure

The data for blood ethanol elimination, mean arterial pressure, and heart rate are given in Table 4. Before the ethanol infusion, baseline blood pressure and heart rate were reduced in BDL rats relative to sham-ligated rats. Infusion of ethanol caused an initial increase in heart rate followed by a slight rise in pressure in BDL rats. The ethanol infusion minimally raised BP in sham-ligated rats but the increase in pressure was not preceded by an increase in heart rate. Thus, it appears that the slight increase in blood pressure in BDL rats may result both from the actions of vasoconstrictive hormones and from an increase in heart rate. Blood pressure gradually declined over the next 2 h, but the difference between BDL and sham-ligated rats persisted.

Blood ethanol concentrations 20, 75 and 135 min after the beginning of the infusion were similar in BDL and sham-ligated rats, and the slopes of the two blood ethanol disappearance curves were identical (−0.75 mg/dl/min).

DISCUSSION

The malaise or abdominal discomfort accompanying BDL and other abdominal surgical procedures may become associated with the flavor of a solution such as alcohol or saccharin to produce a conditioned taste aversion (1,2). However, any of several unconditioned factors may also affect the intake of ethanol after a BDL surgery. For example, (A) the hypotension and renin secretion characteristic of cirrhosis might either stimulate or reduce the ingestion of ethanol (9,11); (B) derangements in neurotransmitters and hormones such as GABA or serotonin (8,16,17) may affect the subsequent ingestion of alcohol (4); (C) a reduced metabolism of ethanol after liver damage may increase the unpleasant intoxicating effects of unmetabolized ethanol and acetaldehyde, and this may diminish the intake of ethanol (15); and (D) an increased sensitivity to a given blood concentration of alcohol may affect the alcohol intake of BDL rats (5).

One way that a conditioned taste aversion may be distinguished from unconditioned effects on intake is the necessary contiguity between the flavor and the onset of the unconditioned stimulus. For instance, aversions are less easily established to familiar flavors than to novel flavors (13), and flavors that are first tasted after the malaise-inducing event are less likely to produce an aversion than flavors that are tasted immediately before the event (3). Unconditioned effects on ethanol

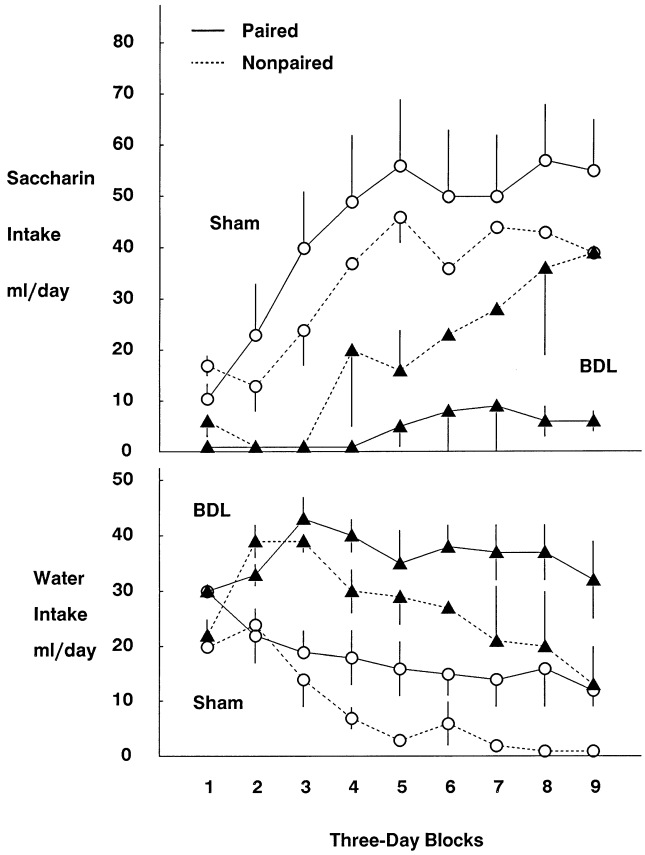

FIG. 6. Daily water and saccharin solution intakes for BDL and sham-ligated rats whose surgery was either paired or not paired with a 2 h bout of saccharin drinking. The paired and nonpaired groups had surgery either immediately or 48 h after access to saccharin, respectively. Means and standard errors of 3-day blocks.

TABLE 4

BLOOD ETHANOL ELIMINATION, BLOOD PRESSURE, AND HEART RATE RESPONSES TO A 1.5 g/kg INTRAVENOUS ETHANOL INFUSION IN BDL AND SHAM-LIGATED RATS

Minutes	BEC	MAP	HR
−10 to 0 (Baseline)			
BDL	—	106 ± 2*	337 ± 7*
Sham	—	121 ± 3	393 ± 11
0 to 15 (Infusion)			
BDL	—	111 ± 2*	388 ± 11
Sham	—	126 ± 2	391 ± 9
15 to 20 (postinfusion, 20 min BEC)			
BDL	154 ± 7	107 ± 2*	378 ± 10
Sham	161 ± 9	120 ± 3	377 ± 9
20 to 75 (75 min BEC)			
BDL	107 ± 4	105 ± 1*	374 ± 6
Sham	116 ± 4	117 ± 2	390 ± 14
75 to 135 (135 min BEC)			
BDL	68 ± 4	101 ± 3*	373 ± 9
Sham	75 ± 5	112 ± 4	389 ± 4

Mean ± SE. Units: blood ethanol concentration (BEC) mg/dl; mean arterial pressure (MAP) mm Hg; heart rate (HR) beats per min.

*$p < 0.05$ vs Sham group.

intake, such as the renin-angiotensin system, GABA and serotonin, reduced metabolism, or increased sensitivity, should not be susceptible to different conditions of contiguity between the surgery and the first taste of ethanol. Similarly, these effects should be selective for ethanol. Unconditioned changes in preferences for various tastants other than alcohol have previously been described in BDL rats (6).

In the present experiments, BDL surgery reduced ethanol or saccharin intake if continuous access to the solution was first provided before or shortly after the surgery. This effect was observed: (A) when ethanol was sweetened with saccharin to increase its palatability; (B) when rats were given about 3 weeks of preexposure to the flavor; and (C) when rats received continuous access to solutions for 5–6 weeks after the surgery. However, providing ethanol or sweetened ethanol for the first time 2 weeks after the BDL surgery eliminated the reduction in intake compared with sham-ligated rats.

The temporal dependence and nonspecificity of the effect of BDL on ethanol or saccharin intake suggests that the effect results from a conditioned aversion to the flavors instead of from unconditioned factors. The most potent aversion demonstrated in this study resulted from an explicit pairing of a robust bout of saccharin ingestion with a BDL surgery 2 h later. Separating the bout of drinking from the surgery by 48 h weakened the effect, as did a continuous access to saccharin for 3 weeks before surgery. Providing ethanol or sweetened ethanol during the 3 weeks of prior access produced somewhat stronger aversions than providing saccharin without ethanol, probably because of the greater palatability of saccharin alone.

Provision of sweetened ethanol for the first time 3 days after BDL surgery also reduced intake relative to sham-ligated rats. Both this group from Experiment 3 and the "nonpaired" saccharin group from Experiment 4, which had a temporary reduction in saccharin intake, seem to challenge the contiguity assumptions of the conditioned taste aversion model. However, we know little of the nature and timing of the unconditioned stimulus after a BDL surgery. Presumably this unconditioned stimulus after BDL surgery is more sustained than the corresponding unconditioned stimulus following a LiCl injection. A novel tastant experienced anytime within the first few days after a BDL surgery might become associated with the accumulating abdominal symptoms.

Potential unconditioned factors that may have affected ethanol intake after BDL include the many hormonal derangements that occur with the disease, as well as a possible reduction of ethanol metabolism or an increased sensitivity to alcohol.

One hormonal system that is activated after BDL surgery, the renin-angiotensin system (21), has been suggested to reduce ethanol intake in rats under several circumstances (reviewed in 11). This effect is not universal (9), but sufficient evidence exists to warrant its consideration. Certain results from the BDL rats in this study are consistent with an activation of the renin-angiotensin system, including a reduced mean arterial blood pressure under baseline and intoxicated conditions and an increased ingestion of water in all experiments. We have also observed an elevation of ad lib water intake under basal maintenance conditions in BDL rats without access to ethanol or saccharin (own unpublished data). Thus, low mean arterial pressure may produce angiotensin formation

in the blood that then increases water intake and reduces ethanol intake.

The most compelling argument against an angiotensin-mediated reduction of ethanol intake in our study is the fact that no such reduction was observed in BDL rats when ethanol or sweetened ethanol were provided two weeks after the surgery instead of before it. Presumably the activation of the renin-angiotensin system would have been similar in the two conditions, yet one condition produced a reduction in ethanol intake and the other did not. Similar arguments could be made against hormonal interactions other than angiotensin, but they all rest on the assumption that the levels of the hormone in the two circumstances are equal, and this has not been measured.

Another unconditioned effect that may have reduced the consumption of ethanol is the metabolic rate. Our experiment did not detect any decline in ethanol elimination after a 15-min infusion of 1.5 g/kg ethanol about one month after BDL or sham-ligation surgery. Even if a difference in ethanol elimination were detected, it is not clear how it could account for the large difference in ethanol intake between BDL rats in the prior access and the delayed access experiments. Presumably a metabolic limit on ethanol consumption (15) would produce the same reduction in ethanol intake regardless of the timing of the access to ethanol unless the access to ethanol itself produced sufficient extra damage to the liver to compromise metabolism. We did not measure ethanol elimination in BDL rats that had been chronically exposed to ethanol. If such an effect of ethanol ingestion on ethanol metabolism exists in BDL rats, it must occur quite rapidly, because ethanol intakes were significantly reduced during the first 4-day block after ligation.

The implications of our data for human health are strongest in the case of a rapidly developing biliary obstruction. The speed of onset of the symptoms were probably important in the potency of the observed aversions, and it is not known how a more slowly developing cirrhosis would affect the ingestion of sapid solutions by rats, much less by humans. Our data do advise careful monitoring of eating habits by patients with biliary obstruction among other diseases (2) since the consequences of the disease may produce potent aversions even to familiar, palatable foods. A cirrhosis-induced aversion to the taste of alcohol in alcoholic patients would be a fortunate consequence of the disease, but the commonness of severe cirrhosis in alcoholics suggests that this does not happen. Enhanced palatability and preexposure to the solutions do appear to blunt the development of an aversion, and BDL rats preexposed to sweetened ethanol continued to consume rather large doses of ethanol daily despite the presumed aversion.

The mortality rates in our experiments were about equal for rats with chronic alcohol treatments (8/47) and without chronic alcohol treatments (6/44). Inspection of the individual data in the ethanol-treated animals that either died or were euthanized revealed several interesting coincidences. One rat that received free selection ethanol treatment for 50 days and died 30 days after BDL surgery had been by far the highest ethanol drinker in his group (23 ml in the first block). Three other rats that had received free selection sweetened ethanol treatment prior to BDL died within 2 weeks of the ligation surgery. These rats collectively had consumed such high quantities of alcohol during the preligation phase that their elimination from the analysis after their deaths produced a statistically significant drop in the average ethanol intake of the group. Only 1 low-to-moderate ethanol drinking rat died in the free selection studies, and this was 41 days after ligation. By contrast, the 3 rats in the forced choice ethanol study that died tended to be among the lowest ethanol drinking rats, possibly because the ligation-induced aversion for ethanol was so strong that it interfered with their ability to maintain normal hydration.

This coincidental mortality in high ethanol consuming rats is consistent with an exacerbation of hepatic disease by alcohol. Interestingly, the dosage of alcohol was voluntary in this instance. Perhaps the combination of alcohol and BDL surgery produced greater hepatic degeneration than either treatment alone, much as ethanol potentiates the hepatic degeneration in rats exposed to carbon tetrachloride (19).

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