DRINKING BEHAVIOR AND SODIUM APPETITE IN AN ANIMAL MODEL OF HEPATIC CIRRHOSIS

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ABSTRACT

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The purpose of this study was to establish the pathophysiology of cirrhosis. An animal mode of bile duct ligation (BDL) was used to assess this process. The BDL rat is an animal model of hepatic cirrhosis that is associated with changes in body fluid balance. The present study consisted of two separate experiments. The first experiment examined changes in drinking behavior in BDL rats versus sham rats following injections of 0.9% physiological saline, 6% hypertonic saline, and isoproterenol. The second experiment examined sodium appetite in BDL rats compared to sham rats. Rats received either BDL or Sham ligation surgery three to four weeks prior to experimental tests. There were no significant differences amid BDL and sham groups in water intake

between the isoproterenol and 6% NaCl. However, the BDL rats did drink significantly more water as compared to sham rats following injections of 0.9% NaCl. A second experiment was conducted with BDL and sham rats. This second experiment examined sodium appetite following a 24 hour fluid/sodium deprivation. Prior to the 24 hour fluid/sodium deprivation the daily average intake in 1.5% NaCl was significantly increased as was total fluid intake. Following the 24 hour fluid/sodium deprivation, water intake was significantly decreased in BDL rats as compared to sham rats; there was a trend for increased 1.5% NaCl intake in BDL rats. Testing drinking behavior allowed me to examine changes in water intake often accompanied with cirrhosis. The second experiment allowed me to examine changes in a specific sodium appetite associated with complications of cirrhosis. Cirrhosis is not only a major health concern, but a leading killer in the United States. These findings can help understand its pathophysiology and establish models of prevention and treatment for this disorder and the accompanying complications.

I. INTRODUCTION

According to the 2006 National Center for Health Statistics, published by the Center for Disease Control (CDC), cirrhosis is the seventh leading cause of death in the United States for individuals between the ages of 25 and 64. According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA, 1997), 15% to 30% of those individuals who drink excessively will develop cirrhosis. A recent article by American Psychological Association (2006), addressed how psychology can play a role in this health care crisis. The article discussed how, predominantly, unhealthy behavior leads to cirrhosis and it being one of the leading causes of death in the United States. The article went on to discuss how psychologists can help solve this health concern, psychologists can help address how to change destructive behaviors, help society drive down costs, and develop prevention and treatment models (Munsey, 2006).

Although there are other causes, such as chronic hepatitis C or other chronic liver diseases, the Mayo foundation in 2006, lists chronic alcohol use as the most common cause of cirrhosis. The problem begins when alcohol is absorbed into the small intestine, and the blood carries it directly into the liver. In the liver the alcohol turns into toxic chemicals which can cause inflammation and tissue injury. The healthy liver begins to form scar tissue from the toxins assaults on the liver and this is proving to be a major problem in the destructive process of the liver. The injured liver eventually is unable to break down the compounds that make up fat. Over time the fat will accumulate and

impair the liver further increasing its susceptibility to injury. In its early stages, the liver becomes enlarged due to the accumulation of fat (fatty liver), but it eventually will shrink as cirrhosis develops. The risk of developing cirrhosis increases with the length of time and the amount of alcohol one drinks.

II. BACKGROUND ON CIRRHOSIS

Liver disease, from heavy alcohol consumption, can occur in moderate drinkers or in one night of binge drinking (Maher, 1997). Excessive alcohol consumption can progress liver disease from a stage of fatty liver into the final stage of cirrhosis. With continued drinking the damage can lead to liver failure and eventually death. While race does not appear to be a risk factor for liver disease gender may. According to a recent study investigating telescoping, the rate of change between use and misuse, they were able to establish a faster course of alcohol dependence in women. Increased telescoping for female's means there is not only a faster progression of the developmental events leading to dependence among female alcoholics but an earlier onset of the adverse consequences (Piazza, Vrbka, & Yeager, 1989). Women are at greater risk for cirrhosis with the consumption of one to two drinks of alcohol a day (20 grams of ethanol), whereas males typically increase their risk with the consumption of five drinks (60 grams of ethanol) a day (Wood, Vinson, & Sher, 2001). Future research ought to examine what the contributing factors are for women and their considerably greater risk. Although metabolism, malnutrition, and genetics may be contributing factors to consider, the mechanisms of hepatic injury are still unclear.

As I mentioned earlier, cirrhosis is a disease of the liver. It is an irreversible, life threatening disease that occurs when scarring damages the liver. The main damage is due to the scarring that occurs from injuries due to assaults on the liver from toxins, such as

alcohol. The scar tissue begins to surround normal liver cells causing nodules to form. When these nodules form they block the small bile duct which causes the bile to back up into the blood stream. How the damage to the liver occurs is not entirely clear. According to The Liver Center in 2003, the progression of liver disease begins with the normal liver progressing to fatty liver, then to liver fibrosis (some scarring), and ends with irreversible scarring and destroying the liver (cirrhosis). Patients with cirrhosis are susceptible to a variety of debilitating complications and their life expectancy is markedly reduced (Maher, 1997). Some of the signs and symptoms of cirrhosis are jaundice (yellowing of the skin and eyes), and encephalopathy (brain damage), ascites (fluid accumulation), hyponatremia (low sodium concentration), coma and eventually death. With cirrhosis factoring in as the seventh leading cause of death in the United States it is an important area for further research and prevention.

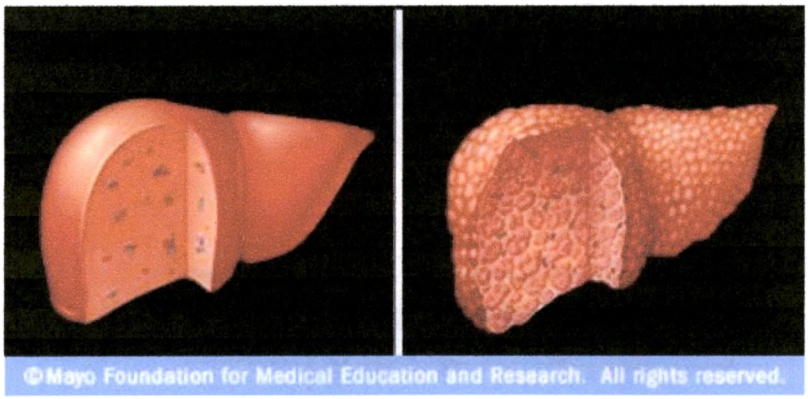

Figure 1. Healthy Liver Versus Cirrhotic Liver.
The left depicts what a normal liver should look like. The image to the right depicts a cirrhotic liver [Used with permission from www.mayoclinic.com, April, 2007].

Cirrhosis is associated with malfunctions in body fluid balance that can lead to ascites or hyponatremia, the result of increased release of vasopressin (AVP) from the posterior pituitary gland. Vasopressin is a polypeptide hormone consisting of nine amino acids. It is synthesized in the hypothalamus in specialized magnocellular cells. It is then transported to the posterior pituitary where it is stored in neurosecretory granules until specific stimuli cause it to be secreted into the blood stream. Increase plasma osmolality and hypovolemia are the principal physiological stimuli for vasopressin secretion, although there are other agents that can induce or inhibit its release (Ferguson, Therapondos, Newby, & Hayes, 2003).

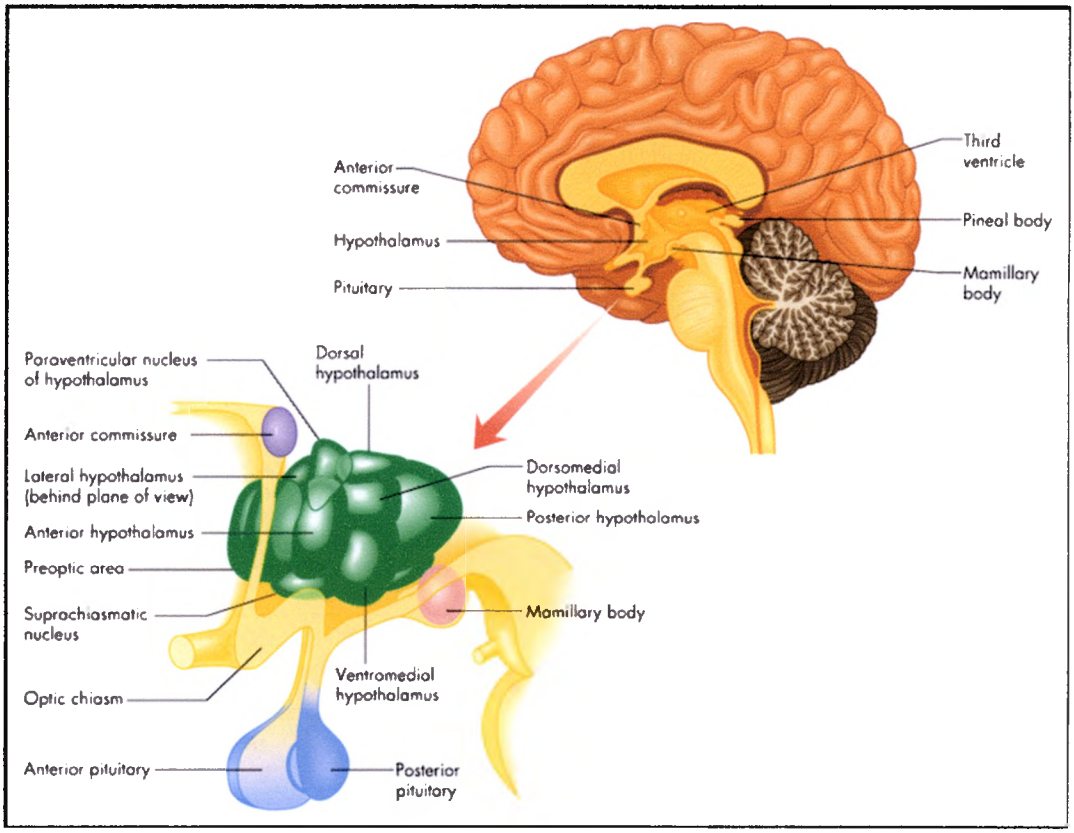

Figure 2. Hypothalamus and Posterior Pituitary Image.
The hypothalamus synthesizes vasopressin while the posterior pituitary stores vasopressin until specific stimuli release it into the bloodstream [obtained from cti.itc.virginia.edu].

The hypothalamus is located at the base of the forebrain below the thalamus and regulates the pituitary gland. The hypothalamus contains several nuclei, however, the paraventricular nucleus (PVN) and supraoptic nucleus (SON) contain the highest concentrations of vasopressin. The human pituitary gland consists of two parts controlled by the hypothalamus, the glandular and the neural. The glandular, or anterior pituitary, includes the release of tropic hormones that control the endocrine glands affecting bodily functions such as, reproduction and the stress response. These hormones also directly affect body organs, such as with bone formation and growth (Rosenzweig, Breedlove, & Watson, 2005). The neural part is the neurohypophysis or the posterior pituitary gland. The neurohypophysis develops from the neuro-ectoderm located in the floor of the third ventricle. The magnocellular neurosecretory axons pass from cell bodies in the SON and PVN of the hypothalamus to the neurohypophysis (posterior pituitary). The posterior pituitary is where the magnocellular neurosecretory granules are stored in the axon terminals. The granules are eventually released when they are stimulated. The peptides from the granules are then able to enter into the bloodstream (Verbalis, 2007).

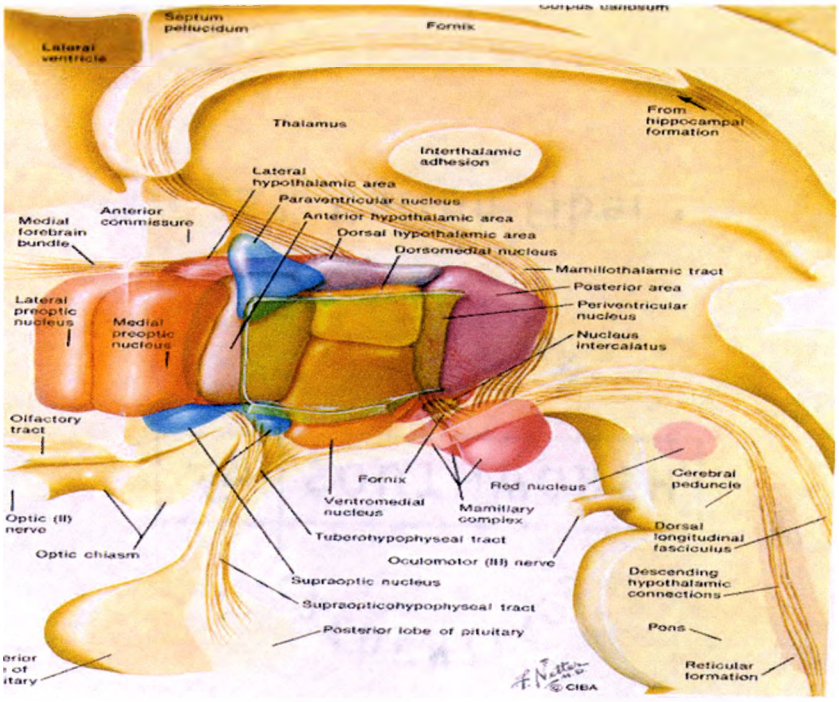

Figure 3. Paraventricular and Supraoptic Nucleus.
These nuclei contain the highest content of vasopressin cells known [obtained from **www.psycheducation.org**].

It is believed that the release of vasopressin is regulated by osmoreceptors and baroreceptors. Even the smallest changes in osmolality (the amount of solutes in the body) can stimulate the receptors of the neurosecretory cells of the hypothalamus. There is a very sensitive linear relationship between plasma osmolality and vasopressin release (Ferguson, Therapondos et al., 2003). The prime determinant in free water excretion in mammals is the regulation of circulating levels of vasopressin in plasma (Verbalis, 2003). Vasopressin constricts blood vessels, raises blood pressure, and reduces excretion of urine. The reduction of urine usually occurs when the body is low on water in an effort to conserve water by concentrating the urine and reducing urine volume. Understanding the different conditions that stimulate vasopressin release is essential for understanding body

fluid homeostasis and complications from a malfunction in the homeostasis. Another consideration is how allostasis has been affected. Allostasis is the body attempting to maintain homeostasis through change. When the allostatic response has experienced an allostatic load and the body is no longer able to recover and return to homeostasis. Chronic challenges from cirrhosis contribute to the allostatic load (McEwen, 2002). For example, when one of the systems involved in body fluid balance is experiences chronic malfunctions another may overload in an attempt to compensate for it. When this response becomes chronic in nature, such as with over expression of vasopressin the system can eventually become damaged and unable to return to body fluid homeostasis.

Body Fluid Homeostasis

With changes in body fluid balance come a multitude of behavioral responses that attempt to minimize the disruption in body fluid volume and composition. There are several physiological (normal bodily functions) and pathophysiological states (disturbance in the biological and physical functions caused by disease states) that are characterized by severe alterations in body fluid homeostasis. For example, during pregnancy plasma volume expands to accommodate perfusion of the placenta and the fetus (Schrier 2006). This volume expansion is associated with changes in fluid intake and fluid retention that are necessary for a healthy pregnancy but that can in some cases produce increased blood pressure preeclampsia. Congestive heart failure is associated with changes in body fluid balance that can lead to increased morbidity and mortality (Schrier, Fassett, Ohara, & Martin, 1998). Liver disease or hepatic cirrhosis is another pathophysiological state that is characterized by impaired body fluid homeostasis.

The human body is composed of approximately 60% water, the largest single element of the body. There are specific balances in the chemical process of the body to maintain necessary bodily functions accurately (Verbalis, 2003). Body fluid homeostasis is the balance between the intake and excretion of water. The two major mechanisms responsible for regulating water metabolism are thirst (e.g. via osmoreceptors) and the posterior pituitary secretion of vasopressin. Thirst is considered to be a defense mechanism against perceived deficits of body fluid. A 1% to 4% increase in plasma osmolality has been shown to lead to the sensation of thirst (Verbalis, 2003). Body fluid volume regulation is a complex process requiring interaction of a variety of efferent (sensory) and neurohumoral efferent (effector) mechanisms (Schrier, Fassett, Ohara, & Martin, 1998). Thirst or water intake and sodium appetite are the principle mechanisms used by the body to replace bodily fluids and solutes that are lost as the result of dehydration (Verbalis, 2003). However, there are times when these mechanisms become ineffective and the system is not operating as it should. One of the main pathophysiololgical states associated with high plasma vasopressin concentrations and water retention is hepatic cirrhosis. Water retention in hepatic cirrhosis is a common problem and can lead to severe disease states such as ascites and hyponatremia.

Ascites

Ascites is the most common complication of cirrhosis and is a state of salt and fluid retention. Previous studies have shown that cirrhotic patients with ascites have an abnormal response to water administration and cirrhotic patients without ascites experience normal water excretion (Schrier, Fassett, Ohara, & Martin, 1998). The most common sign of ascites is a swollen belly due to the accumulation of fluid in the

abdomen. Fever, abdominal pain, and tenderness when pressure is applied to the abdomen indicate that the fluid is infected. With normal retention of fluid individuals adjust their drinking behavior to decrease fluid intake; however, this is not the case in a state. Severe liver disease, such as cirrhosis is associated with this water retention and increase in total body weight such as dilutional hyponatremia, which we will discuss in the following section. Important in understanding ascites and possible prevention models is that once ascites has taken place only half the patients live longer than the following year, according to NIAAA (2004).

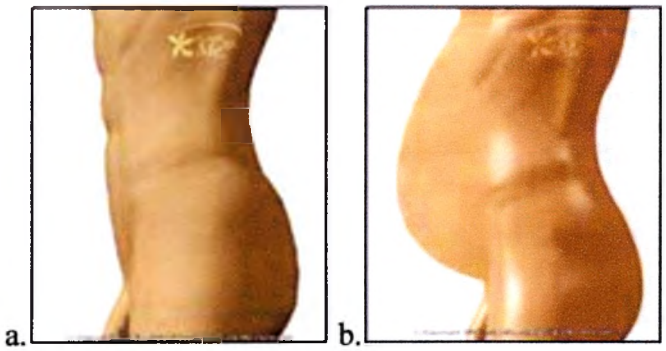

Figure 4. Ascites.

a) Depicts an individual with normal body fluid response, b) depicts an individual who has already developed ascites (fluid retention) [Obtained from **www.ecureme.com**].

Hyponatremia

Hyponatremia is another complication of cirrhosis most likely due to inappropriate vasopressin secretion. Vasopressin secretion increases intravascular volume and dilutes sodium concentrations (Goldsmith, 2005). According to a recent article by Oren in 2005, hyponatremia is the most common electrolyte disorder in the United States.

In previous research by, Krieckhaus (1970), it was established that the rat has an innate need for sodium. Sodium is the only solute a specific appetite has been shown for (expressed as an appetite for chloride salt or NaCl). Most cases of hyponatremia (low sodium levels) are associated with reduced plasma osmolality due to elevated levels of vasopressin release. Severe hyponatremia may cause a shift of water from the plasma into the brain cells. This may lead to cognitive detriments such as confusion, hallucinations, stupors, or coma. Clinical manifestations of hyponatremia are largely neurological in nature. Most of these neurological symptoms are caused by brain edema, where water collects in the brain, as a result of osmotic water movement into the central nervous system (Verbalis, 2007). The severity of neurological dysfunction is highly correlated with the degree of hypoosmolality (Verbalis, 2003).

There are several types of hyponatremia such as, hypervolemic (an increase in blood volume), and hypovolemic (a decrease in blood volume). However, the most common form of hyponatremia is dilutional hyponatremia, a disorder of fluid and electrolyte balance caused by excessive release of vasopressin. Dilutional hyponatremia is a state where the total body water is in excess relative to the sodium stores, which may be low, normal, or increased (Oren, 2005). This change can lead to severe detriments discussed later in this paper. The maintenance of body fluids is carried out within a narrow range made possible by homeostatic mechanisms controlling the intake and excretion of water. A 1% to 2% increase in plasma osmolality stimulates vasopressin release, where a decrease of the same amount suppresses vasopressin. Despite the fact that there is decreased plasma sodium and osmolality, patients with cirrhosis do not have suppressed plasma vasopressin concentrations (Schrier 2006). Hyponatremia is a serious

and life threatening condition which, as we mentioned, can also lead to severE neurological disorders. Mortality rates range from 5% to 50% depending on its severity (Oren, 2005).

The mechanisms responsible for the abnormal body fluid response have been considered, few studies have examined whether changes in water and salt intake contribute to the development of hyponatremia in experimental models of hepatic cirrhosis. Clinical studies, using animals as models, allow us to study and understand cirrhosis and its etiology. It would not be ethical to induce hepatic cirrhosis in a human participant in order to examine the pathophysiology of hepatic cirrhosis; therefore, I used an animal model in my study. Additionally, using an animal model allowed me to account for uncontrollable variables that may be involved in hepatic cirrhosis, such as age, frequency of drinking, diet, and types of alcohol. In addition the rat model allowed me to observe that cirrhosis had developed due to the signs of ascites (see *Ascites*) and jaundice that are consistent in humans with cirrhosis. This was observed by examining the retention of fluid in the belly and by examining the ears and tails of the rats, which begin to turn yellow, due to jaundice. These same signs and symptoms are the same indicators in humans experiencing cirrhosis (see figure 6).

III. BILE DUCT LIGATION BACKGROUND

Recent studies found that BDL rats have increased saline intake, whether they are sodium depleted or not. Fitts, Lane, Starbuck, & Li (1999), suggested that increased sodium appetite in the BDL rats was likely due to central mechanisms affecting the control of the ingestion of water and sodium related to changes in blood volume and pressure. However, this hypothesis was not directly tested in this study. The BDL rats were found to double their saline intake of hypertonic saline on a daily basis three to four weeks after the ligation surgeries. In addition, BDL rats drank more saline then shams [see methods] after a loss of plasma volume that was induced by sodium depletion. However, the mechanisms involved in this process are still unclear and needs further investigating. The study found an increase in the intake of the hypertonic fluid, high concentration of sodium, in the BDL rats after sodium depletion without an elevation of water intake during the repletion stage, or return of water (Fitts, Lane, Starbuck, & Li, 1999). These results are of interest because BDL rats show an increased sodium appetite. Although the previous studies suggest that this is due to changes in blood volume and blood pressure, increased sodium appetite in BDL rats could be an attempt to compensate for the development of dilutional hyponatremia or hypoosmolality (excess water relative to solutes in the extra cellular fluid, also indicates excess total body water relative to total body solutes). Another interesting finding is that the BDL rats have normal water intake despite the fact that they are in a state of hypoosmolality, which should be associated

with decreased water intake. Thus, the changes in body fluid regulation in BDL rats could be the result of altered osmoregulation, an altered ability to keep the body's fluids from becoming too diluted or too concentrated, and not due to changes in blood volume or blood pressure.

In the current studies, I tested the drinking responses of BDL to experimental challenges that selectively produce increased plasma osmolality (hypertonic saline injections) and hypotension (isoproterenol injections). Isoproterenol was chosen for this experiment because it has been shown to induce hypotension thereby eliciting thirst (Fitts, 1994). In addition, I used water deprivation to stimulate a physiological response to water intake and sodium appetite without the use of drugs or the induction of increased hypotension. A net sodium loss occurs during water deprivation as part of the mechanisms that blunts the increase in extracellular hypertonicity and consequently cell dehydration. The concurrent hypovolemia, decreased blood volume, and activation of the renin angiotensin system during water deprivation induce a specific appetite for sodium that is clearly distinct from thirst (Pereira, David, Menani, & DeLuca Jr. 2002). Therefore, the first experiment set out to examine the drinking behavior and how the mechanisms associated with thirst are affected by cirrhosis. The second experiment examined sodium appetite and how changes in plasma osmolality are associated with BDL rats. These were examined to determine if changes in central nervous system mechanisms that regulate thirst and sodium appetite contribute to the development of hyponatremia along with increased plasma AVP.

IV. EXPERIMENTAL DESIGN

The purpose of the following two experiments was to test the hypothesis that changes in salt and water intake are associated with hepatic cirrhosis in animal models. Specifically, experiment one examined changes in experimentally-induced drinking behavior and sodium appetite in bile duct ligated (BDL) rats. In this model, the severing of the passages in the liver that carry bile to the hepatic duct stops the flow of bile to the duodenum and damages the liver over a period of a few weeks (Fitts, Lane, Starbuck, & Li, 1999). This progression leads to the disease state of cirrhosis.

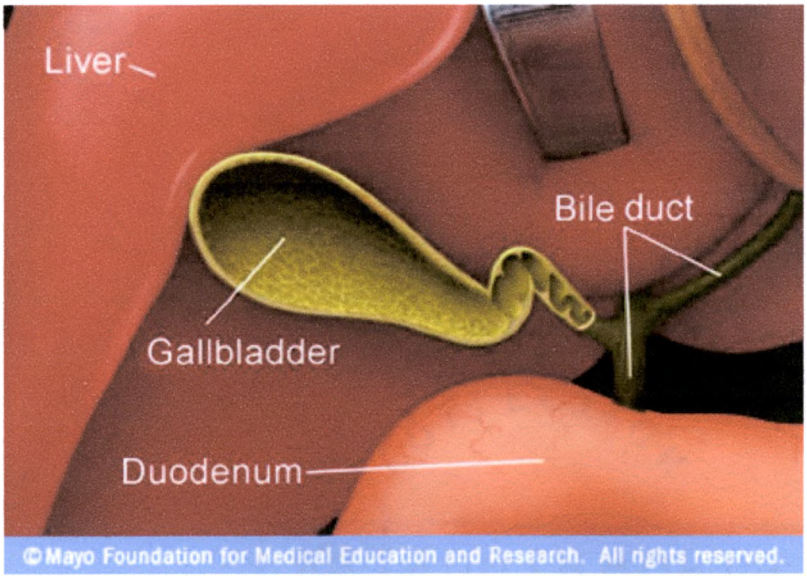

Figure 5. Bile Duct.
The bile severing of the bile duct leads to liver damage over the period of 3-4 weeks in a rat model [Used with permission from mayoclinic.com, April, 2007].

I sought to examine two separate aims in these studies. The first experiment examined drinking behavior of BDL rats as compared to sham rats. I hypothesized that the BDL rats would increase their water intake following physiological and hypertonic saline, and isoproterenol injections despite the fact they were hypoosmotic, as compared to sham rats. Essentially, despite their water retention, it was expected that they would continue to drink as if they were experiencing a state of dehydration as sham rats.

The second experiment examined sodium appetite in BDL rats compared to sham rats. I hypothesized that as the BDL rats continue to drink, they would experience dilutional hyponatremia. The BDL rats and sham rats were given a two bottle choice of 1.5% NaCl (sodium chloride) or water. The BDL rats were expected to increase their intake of 1.5% NaCl (not affecting water intake), as compared to sham rats, in an attempt to increased their sodium levels and bring it back into balance with the increased water baseline.

Methods

Subjects

Subjects were a total of 99 male Sprague-Dawley rats (weight 250-400g, Charles Rivers, MA.), housed in separate cages under controlled temperature and light (12:12h light:dark) conditions to limit any variability between rat housing and to maintain consistency. All the animals were provided access to water and rat chow in their home cages on the days prior to the experimental tests. The animals were randomly assigned to conditions. The number of animals that were in the experimental drinking behavior group totaled 18 (BDL n=8; Sham n=10). Due to the labor intensity associated with BDL processes and time needed for the progression of cirrhosis multiple replication studies

were conducted to measure daily water and 1.5% NaCl intake. The total number of animals in the daily water and 1.5% NaCl intake study were 33 (BDL n= 17; Sham n= 16). The total number of rats used in the 24 hour fluid/sodium deprivation were 25 (BDL n=12 and Sham n= 13).

Procedure

Characterization of BDL Model. An independent validation of BDL as a valid model of cirrhosis with a separate group of 23 rats was conducted. The plasma levels of vasopressin and plasma renin activity were measured to verify that this preparation was valid prior to the water and sodium intake studies. Thirteen rats received BDL surgery using an intraperitoneal (ip) injection of Nembutal anesthesia (65mg/kg ip). An additional ten rats were anesthetized with Nembutal (65 mg/kg ip) and surgically prepared in an identical manner but without actual bile duct ligation. Six weeks following the surgery, each rat was anesthetized with inactin (100 mg/kg ip) and decapitated. Trunk blood was collected into a chilled centrifuge tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Each plasma sample was centrifuged at 10k G for 7 minutes at 4°C. 2 ml of plasma was reserved from each sample and stored at -80°C until it was sent to the radioimmunoassay (RIA) core at the University of Iowa. Plasma vasopressin (AVP) concentrations were measured by specific radioimmunoassay (RIA) following an acetone-petroleum ether extraction (Matsuguchi, Schmid et al., 1981; Sullivan, Cunningham et al., 2003; Ji, Fleming et al., 2005; Gottlieb, Ji et al., 2006). Sensitivity of the AVP RIA is 0.087 pg, which corresponds to 0.170 pg/ml, when factoring in the volume of plasma extracted, extraction efficiency, and volume of extract assayed. The intra- and inter-assay coefficients of variation have averaged 11% and 17% respectively.

Plasma renin activity was determined using a commercially available kit (DiaSorin). A separate sample of whole blood was collected from each rat for measuring plasma osmolality, hematocrit, and plasma proteins. The liver from each rat was harvested to determine the liver weight/body weight ratio as an index of cirrhosis.

Figure 6. Cirrhotic Rat From Lab at UTHSCSA.
Rats with cirrhosis will retain fluid in the abdomen (ascites) and their tail and ears will appear yellow instead of pink (jaundice), this denotes the same symptoms found in the pathology in humans.

Experiment 1: *Drinking behavior experiment.* This study was conducted in three three groups of 6 rats. Each rat received either bile duct ligation or sham ligation surgery. The experimental rats received bile duct ligation (n=8) surgery using Nembutal (65mg/kg ip) anesthesia. The remaining rats (n=10) received sham ligation surgery. These rats were anesthetized with Nembutal (65mg/kg ip) and experienced all the same surgical procedures as the bile duct ligated rats without actual severing the bile duct. Therefore, the sham rats did not develop cirrhosis. All experimental procedures were carried out

during the 12 hour light phase to limit baseline variability in drinking behavior. After a three to four weeks recovery period rats were injected with 0.9% physiological saline (control for stress of injections) or 6% hypertonic saline (induce thirst) subcutaneously (sc) between the skin and underlying tissue. Following injections, rats were placed in metabolic cages where their water intake was recorded for a period lasting 90 minutes, no food was available. At the end of this time each rat was removed from the metabolic cage and returned to their home cage and given two days rest. The recovery period was provided to allow the rat time to recover from the injections. Following the rest period, each group received the opposite injection, this was a restricted randomized counterbalance (if they received 0.9% physiological saline on day one, on day two they received the 6% hypertonic saline injection), and the same process was followed. The rats were given another two day rest period. At the end of the rest period, each rat received an injection of isoproterenol, placed into the metabolic cages, and their water intake was recorded for 90 minutes. They were returned to their home cages following the 90 minute recording stage. On the final day, they randomly received either isoproterenol or 0.9% physiological saline; water was not made available in the metabolic cages for the rats. The rats were euthanized after 90 minutes, to allow for optimal c-fos to peak. Fos is the protein product of the intermediate-early active gene *cfos* a marker for neural activation (Ji, Fleming et al., 2005; Gottlieb, Ji et al., 2006). Each rat was euthanized with an administration of inactin (100 mg/kg ip) and perfused for immunocytochemistry for future research. In a future study, the brains from each rat will be processed for Fos immunohistochemistry in order to determine if the BDL is associated with chronic

activation in the CNS, and if the CNS response to isoproterenol is affected by the BDL, the time course can be seen in figure 7.

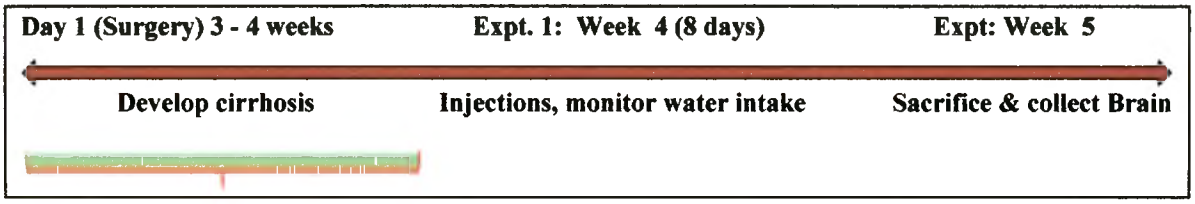

Figure 7. Timeline for Experiment 1. From day 1 of experiment the procedures took approximately 5 weeks each, for 3 groups of rats.

During the perfusion of the rat, a 1-2 ml sample of blood was taken from the heart for measuring plasma osmolality and hematocrit (measure of volume of red blood cells) levels. The liver was dissected from each rat, collected and weighed. The rat's liver weight and body weight were used to calculate a liver weight/body weight ratio in order to verify that the BDL procedure had produced enlargement of the liver that is associated with cirrhosis.

Experiment 2: Sodium appetite experiment. This experiment was conducted in replication. Rats were prepared prior to the experiments by bile duct ligation or sham ligation surgery. Three to four weeks after surgery a two bottle test (consisting of both a 1.5% NaCl bottle and water bottle) was given to each rat in their home cage for one week. The amount of fluid drunk from each bottle was recorded daily for the entire week. The rats were given two days rest following this recording time. At the end of the rest period each rat was challenged with a 24 hour fluid/sodium deprivation (removal of both bottles and food) to stimulate vasopressin release. After the 24 hour deprivation period, the two bottles were returned to each rat in their home cage, in order to monitor and

record intake every half hour, lasting three hours. The rats were given another two days rest at the end of which the two bottles were removed and each rat received an injection of isoproterenol. The rats were decapitated for blood and tissue collection 90 minutes following, time course can be seen in see figure 8.

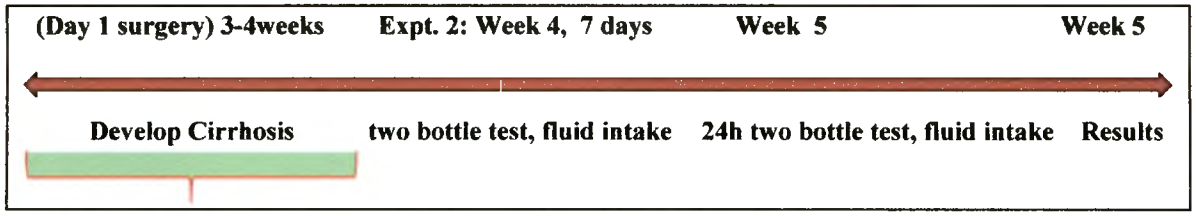

Figure 8. Timeline for Experiment 2. From day 1 of surgeries the continued process for conducting the second experiment took approximately five weeks each, for 3 groups.

Statistical Analyses

In addition to analyzing the experimental groups, I thought it was important to include an analysis of the characteristics of the BDL surgeries. The first experimental group compared drinking behavior between BDL and Sham groups. The second experimental group compared preference for the 1.5% NaCl solution to water intake. Data from the plasma membrane measurements were tested by independent sample *t*-tests (Bruning & Kintz, 1997). Data from experiments 1 and 2 were analyzed using independent sample *t*-tests, BDL vs. Sham (Bruning & Kintz). All values are presented as Mean ± SEM, $p < .05$ was considered statistically significant.

Results

These experiments examined the effect of BDL on drinking behavior and sodium appetite in an animal model. Overall, experiment one found significant increases

associated with water intake in the BDL rats as compared to sham rats in the 0.9% condition. Although we saw increased water intake for the BDL rats for the other tests they were not significant. The second experiment found a significant decrease in water intake in the BDL rat as compared to shams after 24 hour fluid/sodium deprivation. Increased sodium intake was found following the 24 hour fluid/sodium deprivation for the BDL group but it was not significant from the sham rats.

Characterization of BDL Model

At the time that the rats were sacrificed the BDL rats had significantly higher liver weight/body weight ratio compared to shams this indicates that the procedure successfully induced cirrhosis (BDL M = 0.06 ± 0.003; Sham M = 0.03 ± 0.0005; $t(21) = 5.9$, $p < 0.01$). Rats that received BDL also had significantly lower plasma osmolalities than compared to the sham controls (BDL M = 294 ± 1, Sham M = 298 ± 1 mos moles/L; $t(18) = 3.34$, $p < 0.05$) and a significant decrease in hematocrit (BDL M = 42 ± 1%; Sham M = 45 ± 0.7%; $t(21) = 3.5$, $p < 0.05$). Plasma AVP was also significantly higher in the BDL group (BDL M = 36.8 ± 10; Sham = 6.5 ± 2.4 pg/ml; $t(10) = 2.8$, $p < 0.05$). In BDL rats, plasma renin activity was also significantly increased as compared to sham controls (BDL M = 15.8 ± 3.8; Sham M = 6.3 ± 1.4 ng/ml/min; $t(13) = 2.4$, $p < 0.05$).

Experiment 1: Drinking Behavior Experiment: Water Intake Following Hypertonic Saline and Isoproterenol Injections

The first experimental group of BDL and sham rats was examined and analyzed by independent t-tests to compare the differences between groups. It was found that the BDL produced significant increases in plasma vasopressin (Sham M= 6.5 ± 2, BDL M=37 ± 10 pg/ml; $p<0.01$) and plasma renin activity (Sham M=6.3 ± 4, BDL M= 15.8 ± 4ng/ml/min; $p<0.01$). The BDL rats were also significantly hypervolemic with decreased plasma osmolality. Water intake produced by isoproterenol (30 ug/kg, sc) or 6% NaCl (1 ml/100g bw, sc) was not significantly effected by BDL (isoproterenol: Sham M=6.5 ± 1.1; BDL: M= 7.1 ± 2.0 ml; 6% NaCl $t(14) = .278$, $p > 0.05$: Sham M= 8.6 ± .8; BDL M= 10.4 ± 2.6 ml; $t(16) = .592$, $p < 0.05$) (It was necessary to exclude information from two rats because they did not survive until this final test with isoproternenol). However, the BDL rats drank significantly more water compared to sham rats following the injections of 0.9% physiological saline (Sham M=1.86 ± .5; BDL M= 4.8 ± .6 ml); $t(16) = 3.877$, $p < 0.05$). These findings are presented in figure 9.

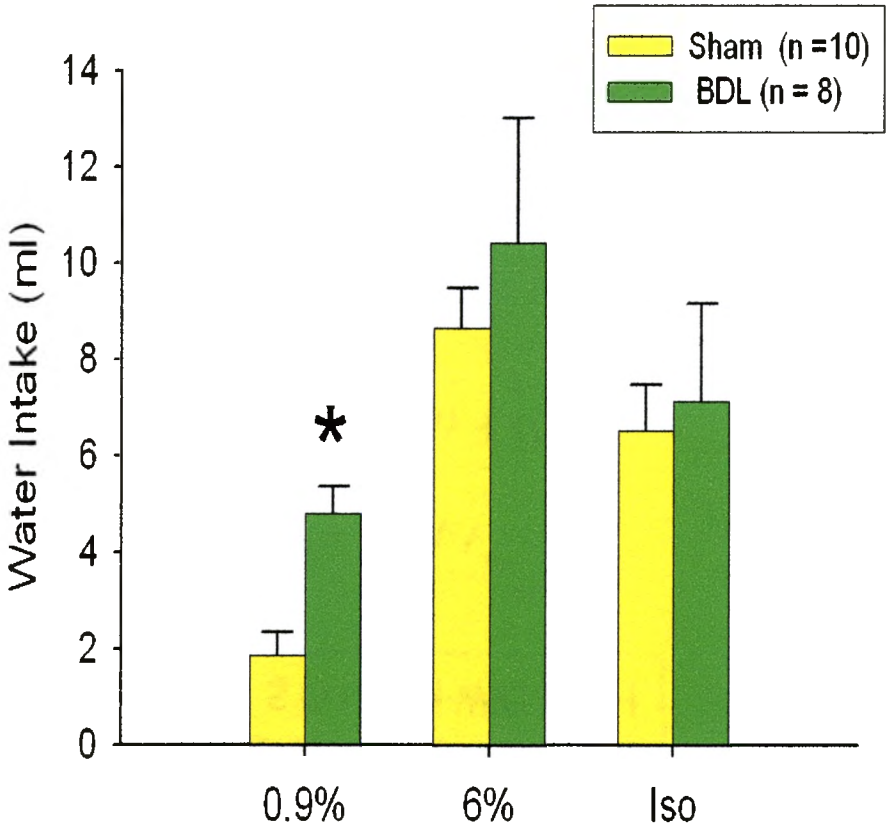

Figure 9. Water Intake.

Figure 9 shows the mean water intake (ml) ± SEM for each group of injections. 0.9% physiological saline does not induce thirst, 6% hypertonic induces thirst osmotically, and isoproterenol induces thirst via ANGII. Bile duct ligated rats (BDL) rats drank significantly more following injections of 0.9% physiological saline compared to shams (surgery without bile duct ligation) (* denotes significant, $p < .05$).

Experiment 2: Sodium Appetite Experiment: Two Bottle Test of Water and 1.5% NaCl Solution.

The second experimental group of BDL and sham rats was examined to compare the sodium solution intake to water intake following a 24 hour fluid/sodium deprivation. Daily water and 1.5% NaCl intake was measured in each group 7 days before the test in the 24 hour fluid/sodium deprivation group of rats and in a second replication group of rats (total of both groups was BDL n= 17; Sham n= 16). The daily intakes for water and 1.5% NaCl were recorded and averaged for each rat, and the between groups comparison was made using a *t*-test for independent groups (BDL vs. Sham). For the total fluid intake the amount of water and 1.5% NaCl were added for each day and averaged. Differences in total fluid intake were tested by *t*-tests for independent groups (BDL vs. Sham). Prior to the 24 hour deprivation test, the daily average 1.5% NaCl intake was significantly increased in BDL rats (sham M=20.0 ± 4, BDL M= 37.7 ± 3 ml: $t(31) = 3.517$, $p < 0.05$) as was the total fluid intake for BDL rats as compared to shams. (Sham M=47.7 ± 5; BDL M= 67.2 ± 5; $p<0.05$), as seen in figure 10. Following the 24 hour fluid/sodium deprivation in the current group and the inclusion of a second replication study (total for both groups was BDL = 13; Sham = 12), the results indicated that water intake was significantly different between the two groups (Sham M=9.99 ± .925, BDL M= 6.351 ± 1.07, $t(23) = 2.559$, $p < 0.05$). However, when I examined the 1.5% NaCl intake separate from the water intake, there was a trend for increased 1.5% NaCl consumption for the BDL rats as compared to the sham rats (Sham M=14.25 ± 2, BDL M= 21.3 ±3, $t(23) = 3.197$, $p > 0.05$). These findings can be seen in figure 11.

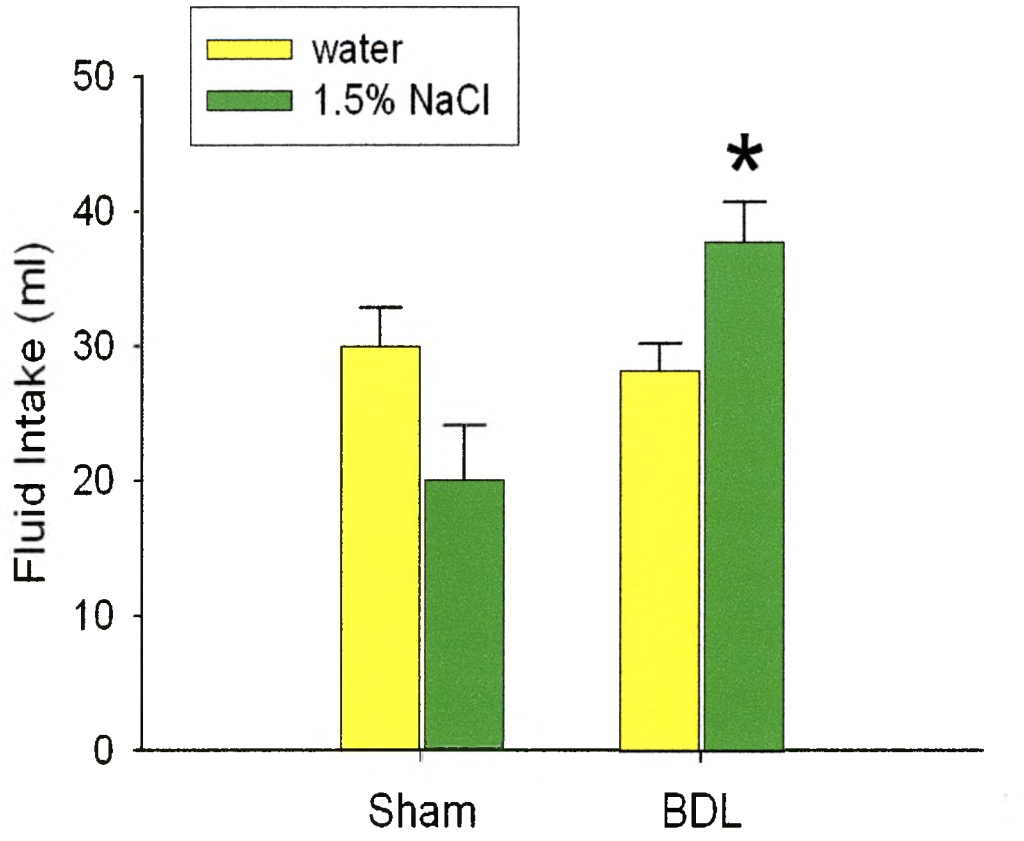

Figure 10. Pre Fluid/Sodium Deprivation Period.

Figure 10 depicts the mean water and sodium intake (ml) ± SEM. The bile duct ligated (BDL) rats exhibit a significant increase in 1.5% NaCl prior to the fluid/sodium deprivation period compared to the sham rats (received surgery without bile duct ligation), intake was recorded for a one week period, (* denotes significant, $p < .05$).

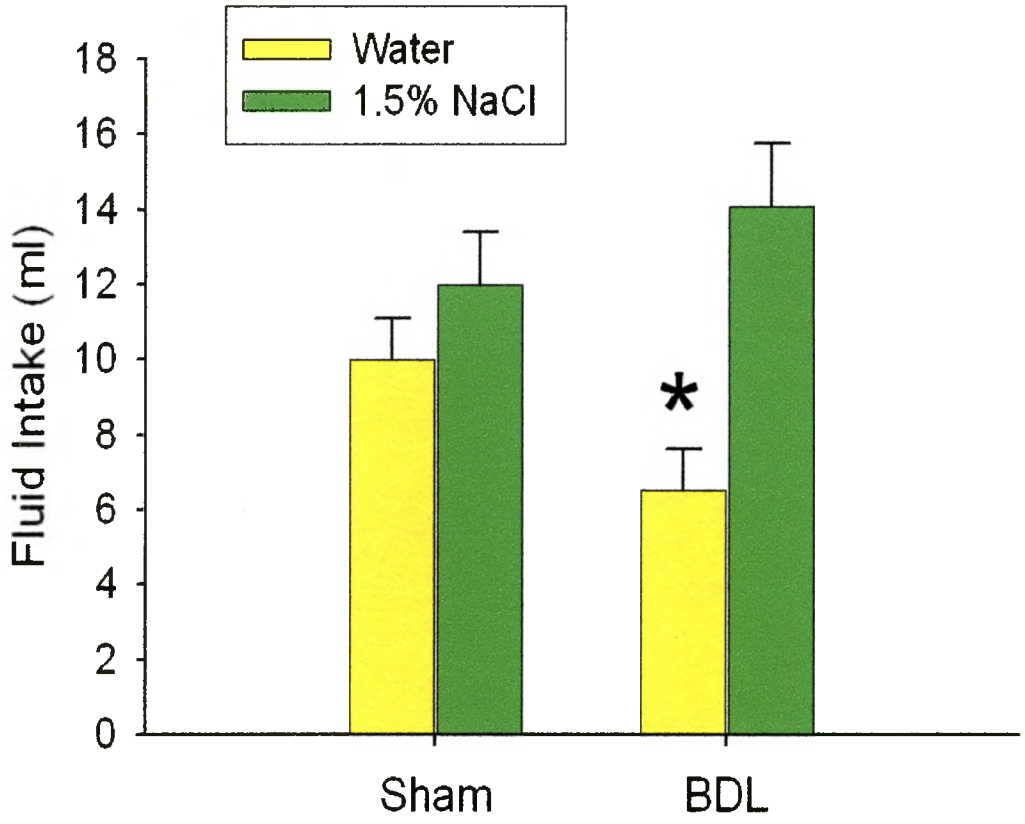

Figure 11. Post 24 hr. Fluid/Sodium Deprivation Period. Water intake was significantly decreased in the BDL group as compared to the shams. The BDL rats exhibit a clear difference in 1.5% NaCl intake compared to sham rats; recorded for 2.5 hours (* denotes significant, $p < .05$).

V. DISCUSSION

The specific goals of the present study were to establish an animal model of hepatic cirrhosis and to test how changes in sodium and water intake contribute to the pathophysiology associated with hepatic cirrhosis. The present research examined whether BDL rats with cirrhosis would experience a need for additional water intake compared to the sham rats. In addition, I examined whether the BDL rats in a 24 fluid/sodium deprived state, given a choice between water and 1.5% NaCl, would experience a preference for the 1.5% NaCl solution, suggesting sodium may be alleviating some of the symptoms associated with hyponatremia.

Rats with chronic BDL had significantly increased basal plasma vasopressin levels and plasma renin activity compared to sham controls. The increased plasma vasopressin likely contributes to the significant changes in plasma osmolality and hematocrit. These observations are consistent with the results of previous studies using the animal model as well as clinical studies in humans with cirrhosis (Wong, Liu, & Blendis, 2001). Further, the results demonstrate the validity of our animal model.

Previous studies have examined whether there were changes in drinking behavior and sodium appetite in association with hepatic cirrhosis in animal models. They found that the BDL rats experienced an increase in their sodium appetite when given access to physiological saline (Fitts, Lane, Starbuck, & Li, 1999). In the current study, we observed that the BDL rats had a significant increase in their daily intake of a more concentrated

saline solution (1.5% NaCl). Following a 24 hour fluid deprivation, BDL rats also showed a significant decrease in water intake as compared to shams while their sodium intake was not significantly different from shams. These results indicate that BDL is associated with significant changes in baseline sodium appetite. Previous studies have suggested that increased activity of the renin-angiotensin system or hypotension is responsible for this increase in activity of the renin-angiotensin system based on the results of the plasma renin activity assay. However, a recent study on patients with hepatic cirrhosis showed that putting these patients on a moderately high sodium diet lowers their plasma renin activity (Wong, Liu & Blendis, 2001). This suppression of the renin angiotensin system was associated with a return to normal sodium balance in patients with cirrhosis. This suggests that the increased sodium appetite in BDL could represent an adaptive response that relieves some of the symptoms associated with cirrhosis. This interpretation opens doors to possible treatments, and if implemented before the ascites takes sets in, a possible model of prevention.

In a separate group of animals, the effects of hypertonic saline and isoproterenol on water intake in BDL rats was tested. Injections of hypertonic saline increases plasma osmolality and stimulate drinking behavior via central nervous system (CNS) osmoreceptors. This experimental paradigm is used as a model of cellular dehydration. In the current study, BDL rats drank more water after injections of physiological saline than the sham controls. When injected with hypertonic saline, the drinking behavior of the BDL rats was not significantly different from sham controls. These results are of interest because the basal plasma osmolality of BDL rats is significantly lower compared to sham controls. This decrease in plasma osmolality would be expected to lead to decreased

drinking behavior in BDL rats following hypertonic saline injections. Based on these observations, additional experiments will be required to determine if hypertonic saline injections in BDL rats produce changes in plasma osmolality that are comparable to the changes observed in sham controls. The changes in osmotically induced drinking behavior observed in the present study could be due to a leftward shift in the relationship between plasma osmolality and water intake. Such a shift could be the result of changes in central osmoreceptor function. Recent studies in our laboratory have shown that BDL is associated with an increase in the expression of TRPV4, a transient receptor potential channel that has been identified as important in osmoreceptor function (Carreno, Ji, & Cunningham, 2007). Increased expression of these proteins in the CNS of rats with BDL could result in an increase in osmotically-induced drinking behavior. Increased drinking behavior could then contribute to the decrease in plasma osmolality and increase plasma volume along with inappropriate vasopressin release.

The antidiuretic hormone vasopressin most certainly plays a role in the pathophysiology of hepatic cirrhosis. Previous research has shown that vasopressin is involved in body fluid homeostasis and is generally released when the body is low on water to conserve the body's store of water (Denton, McKinley, & Weisinger, 1996). The activity of vasopressin-releasing neurons in the hypothalamus is regulated by the same central osmoreceptors as drinking behavior. Results from the current research lead to the possibility that changes in osmoreceptor function could contribute to the inappropriate secretion of vasopressin in cirrhotic rats. Additional research is needed to determine if there is a disruption in osmotic vasopressin secretion and if so, why the mechanisms

involved in vasopressin secretion become affected by the development of hepatic cirrhosis.

In reviewing the current research it appears that the existing models for the treatment of hepatic cirrhosis are able to target the symptoms and complications of cirrhosis, these treatments are proving to be just barely optimal and do not yet focus on prevention. With cirrhosis presenting as one of the leading causes of death in the United States it is important for health professionals to better understand the mechanisms behind the etiology of this disease. Further research is needed in order to better understand the development of cirrhosis and its complications. In addition, experiments that look at what initiates these disruptive behaviors and what could terminate these behaviors may prove to be effective in designing prevention and treatment models.

The direct costs of treating hyponatremia alone, in the United States on an annual basis, are estimated to be between $1.6 billion and $3.6 billion. Treatment of hyponatremia represents a significant healthcare burden (Boscoe, Paramore, & Verbalis, 2006). Hyponatremia occurs in people of all races and ethnicities, with similar prevalence in men and women. The incidence of hyponatremia increases with age for various reasons ranging from age related hormonal changes to medications that promote loss of electrolytes (Oren, 2005). New therapies are needed that may reduce this healthcare burden and minimize costs associated with this condition (Boscoe, Paramore, et al., 2006). New studies are needed to develop these therapies and models of prevention in order to help reduce not only the health care costs but the deleterious effects of liver disease.

Health psychologists can use their insight to help people change their behaviors through education, collaboration with other health professions, and advocating for research funding.

Future experiments should investigate whether the neural mechanisms that produce inappropriate AVP secretion in the BDL rats are contributing to the increased sodium appetite and inappropriate water intake. It is imperative that we establish the mechanisms of action for hepatic cirrhosis and its complications, new models for early intervention and prevention can be developed leading to decreased medical and economic cost and an increased quality of life for patients. However, these experiments are only a stepping stone. Health psychologists are needed to address the importance of finding and designing new treatment and prevention models associated with cirrhosis. Continued research is desperately needed to further establish the pathophysiology of cirrhosis and to help stop, or at the very least, attempt to slow this killer down.

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