Regulation of Secretin in the Neurohypophysis

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ABSTRACT

Secretin (SCT) exists in the hypothalamo-neurohypophysis axis of the rat. Secretin receptor null mice show altered glomerular and renal tubular morphology along with mild polydipsia and polyuria. We hypothesize SCT may play a role in the physiology of water regulation in the rat. Water deprivation (WD) and bile duct ligation (BDL) models were used. Rats were WD for 48 h and rehydrated with either water (RH+W) or 0.9% NaCl (RH+S) for 2 h before sacrifice. Sham and BDL rats were sacrificed 4 weeks after surgery. The presence of SCT and arginine-vasopressin (AVP) in the pituitary; secretin receptor (SCTR), vasopressin receptor 2 (AVP2R), and aquaporin-2 water channel (AQP-2) in the kidney were analyzed using the Western blot technique. Plasma SCT concentrations were measured by enzyme immunoassay (EIA) analysis. Our results show that there is no change in pituitary SCT content in WD rats when compared to controls or in the BDL model. There is also no change in AVP content in either model. No change occurred in plasma SCT concentration for either model. AVP2R and SCTR (at 50kDa) are significantly decreased in the BDL group compared to sham ($P<0.015$; $P<0.004$ respectively). Abundance of glycosylated and non-glycosylated AQP-2 are increased significantly for RH+W and RH+S compared to control ($P<0.002$; $P<0.015$ respectively), but neither showed a difference between sham and BDL groups. We confirm the presence of SCT in the neurohypophysis, but do not support the hypothesis that SCT acts independently of AVP to regulate water absorption in response to homeostatic challenges or disease models with increased AVP. We also show decreased abundance of the SCTR in the kidney of a model of dilutional hyponatremia in the rat.

INTRODUCTION

Secretin (SCT) is known as a gastrointestinal hormone, which regulates pancreas secretion of bicarbonate and potassium ions (9, 17). SCT has been shown to be present throughout the hypothalamus, and recent studies indicate that in the rat it may be released from the posterior pituitary in response to plasma hyperosmolality (12, 13). Immunohistochemical staining reveals that SCT and its receptor are present in the magnocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON), which project to the posterior pituitary (13). The SCT precursor gene is expressed in the brain as well as in the kidney at levels comparable to that of the small intestine (32). Abnormal secretion of SCT from the brain suggests that it has a functional role in some areas of the brain (12). It has been proposed that SCT release from the neurohypophysis may act on the kidney in a manner similar to arginine-vasopressin (AVP) to promote water reabsorption (Diagram 1) (8, 11).

The secretin receptor (SCTR) has been detected in the glomerulus and collecting duct in the developing kidney (35). Mutations of the SCTR gene have been shown to lead to mild polydipsia and polyuria (11). These are common symptoms associated with nephrogenic diabetes insipidus that lead to hyperosmolality. Secretin receptor null mice had altered glomerular and renal tubular morphology, suggesting possible disturbances in the filtration and/or water reabsorption process (11).

Aquaporins, which are expressed in many different tissues, play a role in movement of water across membranes, and are essential for regulating body water homeostasis. The kidney is the main organ responsible for this regulation, and at least seven different aquaporins are expressed at specific sites in the kidney (30, 31).

Aquaporin 2 (AQP-2) is exclusively expressed in the principal cells of the connecting tubule and collecting duct and is the predominant AVP -regulated water channel, which is responsible for regulating the concentration of urine (11, 18, 28, 30, 31). The initial response of renal epithelial cells to activation of vasopressin receptor 2 (AVP2R) by AVP is an increase in cAMP, which regulates trafficking of AQP-2 from intracellular vesicles to the apical membrane of the principal cell (21, 25, 31). Insertion of AQP-2 into the apical membrane leads to water absorption in the collecting duct. It has been shown that the SCTR increases adenylate cyclase activity in the heart and kidney, and thus could trigger a mechanism similar to AVP (3, 8, 10, 34). In addition, a reduction in renal expression of AQP-2 and AQP-4 has been observed in secretin receptor null mice (11).

The syndrome of inappropriate antidiuretic hormone hypersecretion (SIADH) is indicated by 1) hypotonic hyponatremia, 2) urine less than maximally dilute despite plasma hypo-osmolality, 3) increased natriuresis, 4) absence of edema or volume depletion, and 5) normal renal and adrenal function (1). While the most common cause of SIADH is a AVP-secreting tumor, water retention related to disregulation of AVP release is associated with heart and liver failure. In each of these conditions, plasma concentrations of AVP are continually elevated despite hypo-osmolality (19). Type D SIADH is characterized as normal AVP release and response, but abnormal renal expression and translocation of AQP-2 (2, 12). It is possible that in these patients increased plasma SCT could be causing water retention independently of AVP.

However, other studies seem to suggest that the role of SCT in the regulation of water homeostasis may be species specific. Men who were injected with SCT (1unit/kg diluted in 10ml saline) saw a three to fourfold increase in urinary volume and sodium

excretion within the first 30 min after injection. There was a significant rise in urinary pH accompanied by an increase in bicarbonate excretion (39). A second study demonstrated that injected SCT (2CU/kg body weight per h in saline) in men significantly increased urinary water, sodium, and calcium while decreasing free water clearance. It is suggested that the diuretic effect of SCT may impair sodium reabsorption in the renal tubule by an increase in renal plasma flow (RPF), which may be a secondary effect to direct vasodilation of the renal arterioles (40).

In the present studies, we investigated the regulation of SCT in the rat. In some experiments, 48 hours of water deprivation (WD) was used as a systemic progressive homeostatic challenge to physiologically stimulate activation of water retention mechanisms (14). We tested the effects of WD alone or followed by 2 hours access to water (RH+W) or saline (RH+S) (rehydration) on SCT content of the pituitary, SCT release into the plasma, and SCTR regulation in the kidney. In addition, we used an animal model of liver failure that produces dilutional hyponatremia to further investigate the role of SCT in body fluid regulation. Bile duct ligation (BDL) is associated with portal hypertension and systemic hypotension, which is perceived as hypovolemia. AVP is activated by the renin-angiotensin system in response to hypotension despite the hypo-osmotic plasma. This leads to hypervolemia and dilutional hyponatremia (4, 5, 20, 23). In addition, AVP was measured in the pituitary AVP2R and AQP-2 were measured in the kidney.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (300-400 g body wt) were individually housed and maintained in a temperature-controlled environment on a 12:12 light-dark cycle. The rats had ad libitum access to food, water and 0.9% NaCl before the experiments. All experimental protocols were approved by the Institutional Animal Care and Use Committee in accordance with the guidelines of the US Public Health Service, and American Physiological Society.

Water Deprivation

All rats had ad libitum access to food throughout the experiments, except during the 2 h during rehydration before sacrifice. The WD controls were allowed ad libitum access to water throughout the experiment. One group of rats were water deprived for 48 h. Two additional groups were water deprived for 48 h and given water or 0.9% NaCl for 2 h before sacrifice (15). The rats were anesthetized with thiobutabarbital (Inactin; 100mg/kg ip) and immediately decapitated. Trunk blood was rapidly collected into chilled centrifuge tubes containing EDTA. A separate sample was collected into a 1.5-ml microcentrifuge tube that did not contain EDTA. Two hematocrit tubes (Fisher) were filled from the microcentrifuge tube for measurement of hematocrit. The remainder of the blood in the microcentrifuge tube was centrifuged for 5 min (at 10,000 rpm). After the blood was centrifuged, a 200-µl sample of plasma was removed for plasma osmolality measurement using a vapor pressure osmometer (Wescor). The blood collected in tubes containing EDTA was centrifuged at 5,000 rpm for 20 min at 4°C. Plasma was removed

from the tube, placed into a vial, and kept in an ultracold (-80°C) freezer until it was needed for EIA analysis.

Bile Duct Ligation

BDL and sham ligation were performed as previously described (7). The rats were anesthetized with isofluorane (2%). The abdomen was shaved and cleaned. A midline abdominal incision was made and the common bile duct was cut between two ligatures. Any rat showing morbidity or ascites greater than 10% body weight was euthanized. Rats were given food and water ad libitum for 4 weeks after surgery until sacrifice. Sham rats received the same surgical procedure except the bile duct was not ligated or cut. BDL rats and sham ligated controls were anesthetized with thiobutabarbital (Inactin: 100mg/kg jp) and immediately decapitated. Blood and tissue samples were collected as described above.

Western Blot Analysis

Following decapitation, the pituitary was collected, manually homogenized, and sonicated in 0.01g/ml of lysis buffer (0.1% Igepal) supplemented with protease inhibitor for extraction. After Bradford method for protein concentration, total lysate concentration was normalized for all samples and loaded into a 4-20% acrylamide gradient SDS gel (Biorad), electrophoresed in Tris-glycine buffer under denaturing conditions and transferred to a nitrocellulose membrane (Biorad), under semi-dry conditions, in Tris-glycine buffer with 20% methanol. After blocking in TBS-Tween 5% non-fat milk, the membranes were incubated over night with primary antibodies against SCT (1:1000;

Millipore), AVP (1:1000; Millipore), and GAPDH (Millipore). After rinse, the membranes were incubated with HRP conjugated respective secondary antibody (1:10,000; Sigma).

The kidney was collected, manually homogenized, and sonicated in 0.01g/ml of modified lysis buffer containing 1% Triton supplemented with protease inhibitors for extraction. After microplate assay (Biorad) method to determine protein concentration, total lysate concentration was normalized for all samples and loaded onto 4-20% acrylamide gradient SDS gel (Biorad), electrophoresed in Tris-glycine buffer under denaturing conditions and transferred to a nitrocellulose membrane (Biorad), under semi-dry conditions, in Tris-glycine buffer with 20% methanol. After blocking in TBS-Tween 5% non-fat milk, the membranes were incubated overnight with primary antibodies against SCTR (1:500; Abbiotec), AVP2R (1:500; Abcam), AQP-2 (1:500; Sigma), and GAPDH (Millipore). After rinse, the membranes were incubated with HRP conjugated respective secondary antibody (1:10,000; Sigma). The detection system is enhanced chemiluminescence (ECL reagents, Amersham) follow by one of two developing methods. The membrane was either exposed to radiographic film (Hyperfilm ECL, Amersham) or developed digitally using software from Syngene (G:Box, GeneSnap; Syngene). Densitometry of the bands of interest was performed by Image J, and GAPDH was used in normalizing each sample.

Secretin EIA Analysis

Extraction of Protein:

A commercially available enzyme-linked immune absorbance assay was used to measure plasma SCT (Phoenix Pharmaceutical). The plasma (1ml) was mixed with an equal amount of buffer A (TSA and water; Phoenix Pharmaceutical) and centrifuged at 17,000xg for 20 minutes at 4°C for each sample. A SEP-Column (3cc; Waters Sep-Pak) containing 200mg of C18 was equilibrated by washing with buffer B (TSA, acetylnitrile, and water; Phoenix Pharmaceutical) (1ml, once) and then washed with buffer A (3ml, 3 times). The plasma solution was loaded into the pre-equilibrated C-18 SEP-column. The column was slowly washed with buffer A (3ml, twice) and the wash discarded. The peptide was eluted slowly with buffer B (3ml, once) and collected into a polystyrene tube. The eluant was placed into a vacuum centrifuge to evaporate the eluant to dryness. 1x assay buffer was used to reconstitute the dried extract (Phoenix Pharmaceutical).

Assay Procedure:

Fifty µl of standard, plasma, or positive control was added to each well along with 25µl of primary antibody (anti-secretin) and 25µl of biotinylated peptide. The plate was incubated at room temperature (20-23°C) for 2 h. The immunoplate was washed 4 times with 350µl/well of 1x assay buffer. Then, 100µl of SA-HRP solution was added to each well. The plate was incubated at room temperature for 1 h. Following incubation, the immunoplate was washed 4 times with 350µl/well of 1x assay buffer. 100µl of TMB substrate solution was added to each well. The plate was incubated at room temperature for 1 h and 100µl of 2N HCl was added to each well to terminate the reaction (Phoenix

Pharmaceutical). Absorbance O.D. was read at 450nm (Biorad xMark Microplate Spectrophotometer).

Statistics

Data are presented as group means ± one SEM. The data for the WD model was analyzed by one-way analysis of variance with Student Newman-Keuls t-test for post hoc analysis of significant main effects. The data for the BDL model was analyzed by t-test. If assumptions failed, nonparametric tests were performed (Sigma Plot 9.01; 2004). Significance was set at $P<0.05$.

RESULTS

Effects of dehydration on secretin

The results of fluid and weight measurements for the WD experiment are listed in Table 1. Analysis for body weight and plasma osmolality was performed using Kruskal-Wallis ANOVA on Ranks. WD significantly decreased body weight when compared with control ($P<0.05$), but 2 h rehydration with either water or saline significantly increased body weight when compared with WD ($P<0.05$). There was no difference in body weight in either 2 h rehydration group when compared to control. Plasma osmolality measurements were not significantly different between control and WD groups, but RH+W was significantly decreased when compared to WD ($P<0.05$). Also, the average plasma osmolality of rats RH+S was significantly increased when compared to RH+W ($P<0.05$). Hematocrits of WD and RH+W rats were significantly increased when compared to control ($P<0.001$). The hematocrits of RH+W rats were significantly increased compared to WD ($P<0.001$), while hematocrits of RH+S rats were significantly

decreased compared to WD ($P<0.001$). RH+W hematocrit was statistically different from RH+S ($P<0.003$). During the 2 h rehydration period, the rats given access to water drank an average of 27 ± 1.83 ml and the rats given access to saline drank 35 ± 2.52ml. This difference was statistically significant ($P<0.02$).

Pituitaries were subjected to Western blot analysis to evaluate the effects of WD, RH+W, and RH+S on SCT and AVP abundance. Kidneys were subjected to Western blot analysis to evaluate the effects, under the same conditions, on SCTR, AVP2R, and AQP-2 content. Densitometric measurements of the immunoreactive bands were normalized using GAPDH. WD was not associated with significant changes in pituitary SCT content when compared to control (Figure 1). Access to water or saline significantly reduced the content of SCT below control levels (Figure 1). These results indicate that SCT was not affected by WD but was affected by rehydration. There were no significant changes in any of the groups when pituitary AVP was measured (Figure 2).

Plasma was subjected to an EIA assay to evaluate the effects of WD, RH+W, and RH+S on plasma SCT. The data were analyzed with the Kruskal-Wallis ANOVA on Ranks. Plasma SCT levels were not significantly different between any of the four groups (Figure 3).

Similar results were observed in the kidney. Two bands were observed in the Western blot analysis for the SCTR (Figure 4). The data sheet from Abbiotec stated that SCTR is found at 50kDa. Long et al. described SCTR to be around 64kDa (24). The SCTR 50kDa band was analyzed using the Kruskal-Wallis ANOVA on Ranks. Neither band showed significant differences among the groups. The two bands were added together in order to analyze the total SCTR abundance using the Kruskal-Wallis ANOVA

on Ranks. This showed a significant increase in content for the RH+W group compared to the control. There were no other differences (Figure 5). AVP2R content, analyzed using the Kruskal-Wallis ANOVA on Ranks, did not show a change (Figure 6) (37, 41). Both glycosylated and non-glycosylated content of AQP-2 were analyzed (22). No significant difference appeared between WD and control for either glycosylated or non-glycosylated AQP-2, but both rehydration groups were different from control for glycosylated and non-glycosylated AQP-2 (Figure 7). This pattern continued when the two bands were added together in order to analyze the total AQP-2 abundance (Figure 8).

Hepatic cirrhosis and secretin

BDL was performed to determine if SCT would function similarly to AVP in a different model of osmotic challenge. The results of fluid and weight measurements for BDL rats and sham-operated controls are listed in Table 2. The liver/body ratio for BDL rats was significantly increased when compared to sham ($P<0.001$). However, there were no significant differences between sham and BDL rats concerning body weight, osmolality, and hematocrit. This suggests that BDL was associated with liver damage but not significant water retention in this group of rats.

The pituitaries and kidneys were subjected to Western blot analysis, as previously described, for the same hormones, receptors, and channel. SCT content in the pituitary of BDL rats showed no significant difference to that of sham rats (Figure 9). These results further suggest that SCT may not have contributed to water retention in BDL rats. AVP in this model was analyzed using the Mann-Whitney Rank Sum test. AVP content in the pituitary showed no difference between BDL and sham groups (Figure 10). The SCTR

content at 64kDa displayed no difference between sham and BDL groups, but bands at 50kDa showed a significant decrease of SCTR abundance in the kidneys of BDL rats compared to sham operated controls (Figure 11 and 12). Both bands were added together and the total SCTR content for the BDL was significantly decreased when compared to sham (Figure 13) when using the Mann-Whitney Rank Sum Test. AVP2R abundance also showed a significant decrease associated with BDL compared to sham (Figure 14). The renal content of non-glycosylated AQP-2 in this model was analyzed using the Mann-Whitney Rank Sum test. AQP-2 abundance in the kidney was not different between sham and BDL groups for either glycosylated or non-glycosylated bands (Figure 15). Total AQP-2 abundance showed no difference between the groups as well (Figure 16).

Also, preliminary data suggest that there were no changes in circulating plasma SCT concentrations in the BDL group when compared to sham operated controls (Figure 17).

DISCUSSION

It is well known that AVP is the primary hormone involved in water retention. AVP release is regulated by blood osmolality and may be altered by changes in blood volume (14). AVP, which is synthesized in the SON and PVN, stored and released by the posterior pituitary, can act on the renal AVP2R. This receptor is present in principal cells of the kidney, and when stimulated activates the upregulation and insertion of AQP-2 water channels into the apical membranes of the collecting tubule principal cells via cAMP (27, 38). Our results show that there is no change in pituitary AVP during dehydration. AVP2R expression in the kidney did not change for WD or rehydration

groups. This measurement is not specific to active AVP2R in the basolateral membrane of the collecting duct since the whole kidney was homogenized for the Western blot analysis and AVP2R is also present in the distal convoluted tubule. It could be possible that AVP2R in the membrane, vesicles, or in the middle of synthesis were all combined in this analysis. This may also be true for the AQP-2 water channels. AQP-2 can be found the apical plasma membrane and apical vesicles of the collecting duct as well as in the connecting tubule (31). Our data shows two distinct bands for AQP-2 corresponding to non-glycosylated and glycosylated AQP-2 (22). In both cases AQP-2 content was not affected by WD but significantly increased following 2 h rehydration with either water or saline. This was also the case when both bands were combined to analyze total AQP-2 abundance.

Previous work in the rat has indicated that SCT may play a role in the hypothalamo-neurohypophyseal axis and renal system in order to regulate water homeostasis. In the rat, the SCT precursor gene has been shown to be present in the brain and kidneys as well as other tissues (32). During plasma hyperosmolality in rats, SCT was released from the posterior pituitary and SCT plasma levels were elevated (12). Transcripts from SCTR were found in the glomerulus and collecting duct in the developing kidney from mice embryos (35). Secretin receptor-null mice were accompanied by altered glomerular and renal tubular morphology along with mild polydipsia and polyuria, suggesting SCT plays a significant role in renal function (11).

However, results from other studies suggest that the role of SCT in the regulation of water homeostasis may be species specific. SCT has been shown to regulate water transportation in rat cholangiocytes via aquaporin 1 water channels (26) and may,

through another system, regulate AQP-2 water channels. In contrast to that observed in rats and mice, injection of SCT into men showed an opposite effect on the renal system, resulting in increased urinary volume, and sodium excretion (39, 40). These results suggest in humans SCT has a diuretic effect in contrast to the antidiuretic effects observed in the mouse.

In order to better understand the possible role of SCT in water homeostasis in the rat, we examined the effects of 48 h WD and 2 h rehydration of either water or saline on the abundance of SCT in the pituitary and plasma. Our results indicate that WD is not associated with changes in SCT pituitary content, although rehydration with either water or saline did significantly decrease pituitary SCT. Plasma SCT levels were unchanged for all four groups suggesting that if SCT is released from the posterior pituitary, it may not contribute significantly to plasma SCT concentrations. SCT released from the gastrointestinal tract may have a primary influence on plasma concentration.

SCTR abundance in the kidney was analyzed and two distinct bands were found. One band corresponded with the Abbiotec anti-SCTR antibody data sheet, which stated a weight of 50kDa. The other band corresponded with Long et al. who published a weight of 64kDa (24). It is quite possible that the band at 64kDa may be glycosylated while the band at 50kDa is non-glycosylated, but we are unable to confirm this at this time. Our results show no change in SCTR content (for either band) between any groups. However, when both bands are analyzed together for total SCTR content RH+W is significantly increased when compared to control. It does not appear that SCT plays a AVP-like role during WD.

In order to determine if SCT could contribute to dilutional hyponatremia in a manner similar to AVP, the BDL model of cirrhosis was performed in the rat (4). BDL induces liver cirrhosis, which leads to portal hypertension and systemic hypotension. This functional change in blood volume has been proposed to cause an increase in circulating AVP, elevated cardiac output, and increased splanchnic arterial vasodilation. AVP binds to AVP2R in the collecting duct of the kidney, increasing water absorption, and leads to the development of dilutional hyponatremia (6, 16). Data from Table 2 reveals that the BDL group does in fact have liver damage as shown by the significant increase of the liver/body weight ratio for BDL compared to sham. However, it has not reached the point to cause significant water retention since there was no change in osmolality and hematocrit. Therefore, we failed to observe a significant change in AVP stored in the pituitary. Our samples were analyzed 4 weeks after surgery, which may not have allowed appropriate time to activate the renin-angiotensin system (RAS) although we did observe these changes at 4 weeks in previous work with this model (7). The results show no change in SCT content in the pituitary four weeks after BDL surgery. In addition, preliminary data measuring plasma SCT concentration in sham and BDL rats suggest there is no change as well. This would suggest that even though there is liver damage, it was not associated with significant changes in SCT.

AQP-2 in the kidneys showed a decreased expression as cirrhosis progresses. In addition, the receptors become desensitized to AVP by increased cAMP phosphodiesterase activity (5). It is also plausible that the AVP2R no longer responds to the excess of AVP, a mechanism aimed at avoiding dilution hyponatremia. AQP-2 content, in both non-glycosylated and glycosylated forms, showed no change between

sham and BDL groups, nor was there a change in total AQP-2 content. Our results did show that AVP2R content in the kidney is significantly decreased following BDL (Diagram 2). This offers another possible explanation for the lack of water retention in BDL rats in the present study.

Similarly, the 50kDa form of the SCTR is significantly decreased in BDL rats when compared to sham operated controls. Interestingly, the abundance of the 64kDa form of SCTR shows no change. Together the total SCTR content for BDL was significantly decreased when compared to sham. This result was mainly due to a decrease in the 50kDa form. The SCTR has been shown to be glycosylated, which would account for the difference in molecular weights and possibly why the abundance of these two forms of the receptor could be differentially regulated. Glycosylation of the SCTR is essential for its function in humans. Mutations at two glycosylation positions have been shown to affect the function of the receptor by changing its structure. Glycosylation of this receptor is required to maintain a high affinity binding state (33). It may also be possible that, during the early stages of cirrhosis, the liver sends a signal that influences the mechanism that decreases synthesis or increases degradation of SCTR in the kidney that does not influence its glycosylation. This would explain why there is no change in the higher molecular weight form of SCTR while the total amount of SCTR is decreased in BDL. If the BDL was allowed to continue, the amount of SCTR might have decreased even further. This could result in a decrease in glycosylated SCTR since the overall amount of SCTR has decreased to the point where the availability of the SCTR becomes the rate-limiting step for glycosylation. The two different bands may represent two different SCTR subtypes that may be from a single gene product (Diagram 2).

Posttranslational processing of the SCTR has been shown to alter patterns of SCT binding and activation. There are two molecular forms of SCT: fully processed SCT and its immediate precursor, secretin-Gly. It is plausible that different SCTR subtypes exist in order to select between SCT and secretin-Gly (36). Liver damage may send a signal to decrease the amount of one receptor subtype (50kDa) while leaving the other subtype (64kDa) unchanged.

Type D SIADH shows all the same signs and symptoms as SIADH, but the key difference is that plasma AVP is normal (2, 12, 29), thus, leading many to believe that another hormone may have the same effects on the renal system as does AVP. We can confirm the presence of SCT in the neurohypophysis. Our study, however, does not support the hypothesis that neurohypophysial SCT is regulated by WD or BDL in the rat. If SCT does influence renal function in the rat, it does not play a role similar to that reported in the mouse or may not be related to neurohypophyseal secretin. We can also conclude that liver damage affects the amount of SCTR in the kidney of the rat. However, these effects are specific to a 50kDa form of the receptor. These changes in renal SCTR content could alter function associated with hepatic cirrhosis. Further examination is needed to understand the mechanism.

Table 1: Fluid and body weight measurements amongst dehydration groups.

Group	Body Weight (g)	Osmolality (mOsm/kg)	Hematocrit (%)
Control	342 ± 8.27 (11)	302 ± 2.4 (10)	46 ± 0.39 (9)
48hWD	300 ± 4.85 (13) [a]	315 ± 6.7 (8)	54 ± 0.62 (10) [a]
2h Rehydrated-Water	345 ± 15.7 (8) [b]	285 ± 2.25 (6) [b]	50 ± 1.06 (8) [a,b]
2h Rehydrated-Saline	331 ± 5.19 (9) [b]	307 ± 1.9 (9) [c]	47 ± 0.32 (9) [b,c]

Data are mean ± SEM. [a] significantly different from control; [b] significantly different from 48hWD, [c] significantly different from 2h Rehydrated-Water.

Table 2: Fluid and weight measurements amongst BDL groups.

Group	Body Weight (g)	Osmolality (mOsm/kg)	Hematocrit (%)	Liver/Body Ratio
Sham	456.7 ± 23.8 (9)	308 ± 3.52 (9)	48 ± 1.54 (9)	0.039 ± 0.002 (9)
BDL	411 ± 15.9 (11)	306 ± 3.49 (11)	47 ± 1.17 (11)	0.079±0.003 (11) *

Data are mean ± SEM. * significantly different from sham.

REFERENCES

1. **Bartter FC and Schwartz WB.** The syndrome of inappropriate secretion of antidiuretic hormone. *Am.J.Med.* 42: 5: 790-806, 1967.

2. **Baylis PH.** The syndrome of inappropriate antidiuretic hormone secretion. *Int.J.Biochem.Cell Biol.* 35: 11: 1495-1499, 2003.

3. **Bell D and McDermott BJ.** Secretin and vasoactive intestinal peptide are potent stimulants of cellular contraction and accumulation of cyclic AMP in rat ventricular cardiomyocytes. *J.Cardiovasc.Pharmacol.* 23: 6: 959-69, 1994.

4. **Better OS and Massry SG.** Effect of chronic bile duct obstruction on renal handling of salt and water. *J.Clin.Invest.* 51: 2: 402-411, 1972.

5. **Brond L, Hadrup H, Salling N, Torp M, Graebe M, Christensen S, Nielsen S and Jonassen TE.** Uncoupling of vasopressin signaling in collecting ducts from rats with CBL-induced liver cirrhosis. *Am.J.Physiol.* 287: 4: F806-815, 2004.

6. **Cardenas A and Arroyo V.** Mechanisms of water and sodium retention in cirrhosis and the pathogenesis of ascites. *Best Pract.Res.Clin.Endocrinol.Metab.* 17: 4: 607-622, 2003.

7. **Carreno FR, Ji LL and Cunningham JT.** Altered central TRPV4 expression and lipid raft association related to inappropriate vasopressin secretion in cirrhotic rats. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 296: 2: R454-66, 2009.

8. **Charlton CG, Quirion R, Handelmann GE, Miller RL, Jensen RT, Finkel MS and O'Donohue TL.** Secretin receptors in the rat kidney: adenylate cyclase activation and renal effects. *Peptides.* 7: 5: 865-871, 1986.

9. **Chow BK.** Molecular cloning and functional characterization of a human secretin receptor. *Biochem.Biophys.Res.Commun.* 212: 1: 204-211, 1995.

10. **Christophe J, Waelbroeck M, Chatelain P and Robberecht P.** Heart receptors for VIP, PHI and secretin are able to activate adenylate cyclase and to mediate inotropic and chronotropic effects. Species variations and physiopathology. *Peptides* 5: 2: 341-353, 1984.

11. **Chu JY, Chung SC, Lam AK, Tam S, Chung SK and Chow BK.** Phenotypes developed in secretin receptor-null mice indicated a role for secretin in regulating renal water reabsorption. *Mol.Cell.Biol.* 27: 7: 2499-2511, 2007.

12. **Chu JY, Lee LT, Lai CH, Vaudry H, Chan YS, Yung WH and Chow BK.** Secretin as a neurohypophysial factor regulating body water homeostasis. *Proc.Natl.Acad.Sci.U.S.A.* 106: 37: 15961-15966, 2009.

13. **Chu JY, Yung WH and Chow BK.** Endogenous release of secretin from the hypothalamus. *Ann.N.Y.Acad.Sci.* 1070: 196-200, 2006.

14. **Dunn FL, Brennan TJ, Nelson AE and Robertson GL.** The role of blood osmolality and volume in regulating vasopressin secretion in the rat. *J.Clin.Invest.* 52: 12: 3212-3219, 1973.

15. **Gottlieb HB, Ji LL, Jones H, Penny ML, Fleming T and Cunningham JT.** Differential effects of water and saline intake on water deprivation-induced c-Fos staining in the rat. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 290: 5: R1251-61, 2006.

16. **Guevara M and Gines P.** Hyponatremia in liver cirrhosis: pathogenesis and treatment. *Endocrinol Nutr.* 57: 2: 15-21, 2010.

17. **Ho PK, Fong RS, Kai HS, Lau EH, Ngan ES, Cotton CU and Chow BK.** The human secretin receptor gene: genomic organization and promoter characterization. *FEBS Lett.* 455: 3: 209-214, 1999.

18. **Hoffert J, Chou C and Knepper MA.** Aquaporin-2 in the "-omics" Era*. *J Biol Chem.* 284: 22: 14683-14687, 2009.

19. **Ishikawa S, Saito T and Kasono K.** Pathological role of aquaporin-2 in impaired water excretion and hyponatremia. *J.Neuroendocrinol.* 16: 4: 293-296, 2004.

20. **Jonassen TE, Heide AM, Janjua NR and Christensen S.** Collecting duct function in liver cirrhotic rats with early sodium retention. *Acta Physiol.Scand.* 175: 3: 237-244, 2002.

21. **Kamsteeg EJ, Savelkoul PJ, Hendriks G, Konings IB, Nivillac NM, Lagendijk AK, van der Sluijs P and Deen PM.** Missorting of the Aquaporin-2 mutant E258K to multivesicular bodies/lysosomes in dominant NDI is associated with its monoubiquitination and increased phosphorylation by PKC but is due to the loss of E258. *Pflugers Arch.* 455: 6: 1041-1054, 2008.

22. **Kim SW, Jeon YS, Lee JU, Kang DG, Kook H, Ahn KY, Kim SZ, Cho KW, Kim NH, Han JS and Choi KC.** Diminished adenylate cyclase activity and aquaporin 2 expression in acute renal failure rats. *Kidney Int.* 57: 4: 1643-1650, 2000.

23. **Li Y, Song D, Zhang Y and Lee SS.** Effect of neonatal capsaicin treatment on haemodynamics and renal function in cirrhotic rats. *Gut* 52: 2: 293-299, 2003.

24. **Long SH, Berna MJ, Thill M, Pace A, Pradhan TK, Hoffmann KM, Serrano J and Jensen RT.** Secretin-Receptor and Secretin-Receptor-Variant Expression in Gastrinomas: Correlation with Clinical

and Tumoral Features and Secretin and Calcium

Provocative Test Results. *J Clin Endocrinol Metab.* 92: 11: 4394-4402, 2007.

25. **Lorenz D, Krylov A, Hahm D, Hagen V, Rosenthal W, Pohl P and Maric K.** Cyclic AMP is sufficient for triggering the exocytic recruitment of aquaporin-2 in renal epithelial cells. *EMBO Rep.* 4: 1: 88-93, 2003.

26. **Marinelli RA, Pham L, Agre P and LaRusso NF.** Secretin promotes osmotic water transport in rat cholangiocytes by increasing aquaporin-1 water channels in plasma membrane. Evidence for a secretin-induced vesicular translocation of aquaporin-1. *J.Biol.Chem.* 272: 20: 12984-12988, 1997.

27. **Matsumura Y, Uchida S, Rai T, Sasaki S and Marumo F.** Transcriptional regulation of aquaporin-2 water channel gene by cAMP. *J.Am.Soc.Nephrol.* 8: 6: 861-867, 1997.

28. **Morello JP, Salahpour A, Petaja-Repo UE, Laperriere A, Lonergan M, Arthus MF, Nabi IR, Bichet DG and Bouvier M.** Association of calnexin with wild type and mutant AVPR2 that causes nephrogenic diabetes insipidus. *Biochemistry* 40: 23: 6766-6775, 2001.

29. **Multz AS.** Vasopressin dysregulation and hyponatremia in hospitalized patients. *J.Intensive Care Med.* 22: 4: 216-223, 2007.

30. **Nejsum LN.** The renal plumbing system: aquaporin water channels. *Cell Mol.Life Sci.* 62: 15: 1692-1706, 2005.

31. **Nielsen S, Frokiaer J, Marples D, Kwon TH, Agre P and Knepper MA.** Aquaporins in the kidney: from molecules to medicine. *Physiol.Rev.* 82: 1: 205-244, 2002.

32. **Ohta M.** Tissue-specific expression of the rat secretin precursor gene. *Biochemical & Biophysical Research Communications* 183: 2: 390, 1992.

33. **Pang RT, Ng SS, Cheng CH, Holtmann MH, Miller LJ and Chow BK.** Role of N-linked glycosylation on the function and expression of the human secretin receptor. *Endocrinology* 140: 11: 5102-5111, 1999.

34. **Robberecht P, Waelbroeck M, Noyer M, Chatelain P, De Neef P, Konig W and Christophe J.** Characterization of secretin and vasoactive intestinal peptide receptors in rat pancreatic plasma membranes using the native peptides, secretin-(7-27) and five secretin analogues. *Digestion* 23: 3: 201-210, 1982.

35. **Siu FK, Sham MH and Chow BK.** The prenatal expression of secretin receptor. *Ann.N.Y.Acad.Sci.* 1070: 561-565, 2006.

36. **Solomon TE, Varga G, Zeng N, Wu SV, Walsh JH and Reeve JR,Jr.** Different actions of secretin and Gly-extened secretin predict secretin receptor subtypes. *Am.J.Physiol.Gastrointest.Liver Physiol.* 280: 1: G88-94, 2001.

37. **Sonntag M, Wang MH, Huang MH and Wong NLM.** Endothelin Upregulates the Expression of Vasopressin V2 mRNA in the Inner Medullary Collecting Duct of the Rat. *Metabolism.* 53: 9: 1177-1183, 2004.

38. **Verbalis JG.** Disorders of body water homeostasis. *Best Practice & Research Clinical Endocrinology & Metabolism.* 17: 4: 471, 2003.

39. **Viteri AL, Poppell JW, Lasater JM and Dyck WP.** Renal response to secretin. *J.Appl.Physiol.* 38: 4: 661-664, 1975.

40. **Waldum HL, Sundsfjord JA, Aanstad U and Burhol PG.** The effect of secretin on renal haemodynamics in man. *Scand.J.Clin.Lab.Invest.* 40: 5: 475-478, 1980.

41. **Zhu X and Wess J.** Truncated V2 Vasopressin Receptors as Negative Regulators of Wild-Type V2 Receptor Function. *Biochemistry.* 37: 45: 15773-15784, 1998.

Diagram 1

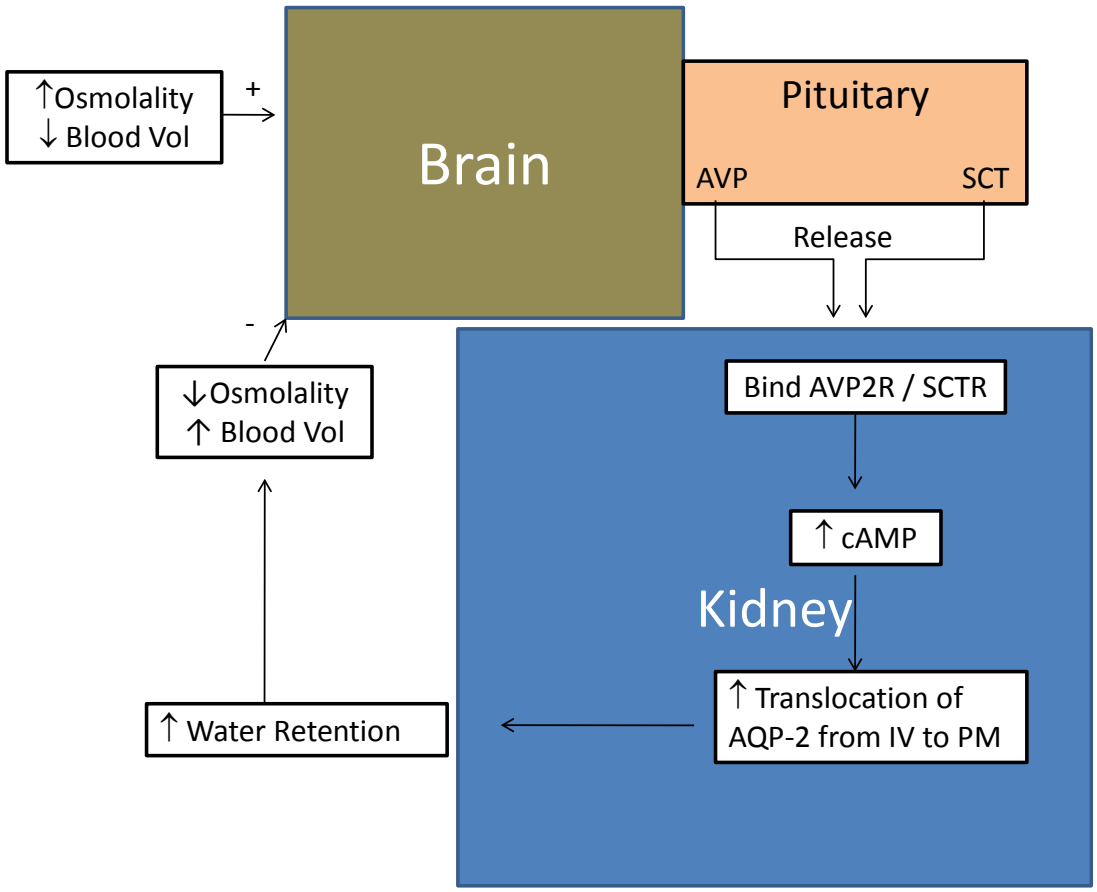

↑Osmolality
↓ Blood Vol
+
Brain
Pituitary
AVP
SCT
Release
-
↓Osmolality
↑ Blood Vol
Bind AVP2R / SCTR
↑ cAMP
Kidney
↑ Translocation of AQP-2 from IV to PM
↑ Water Retention

Figure 1

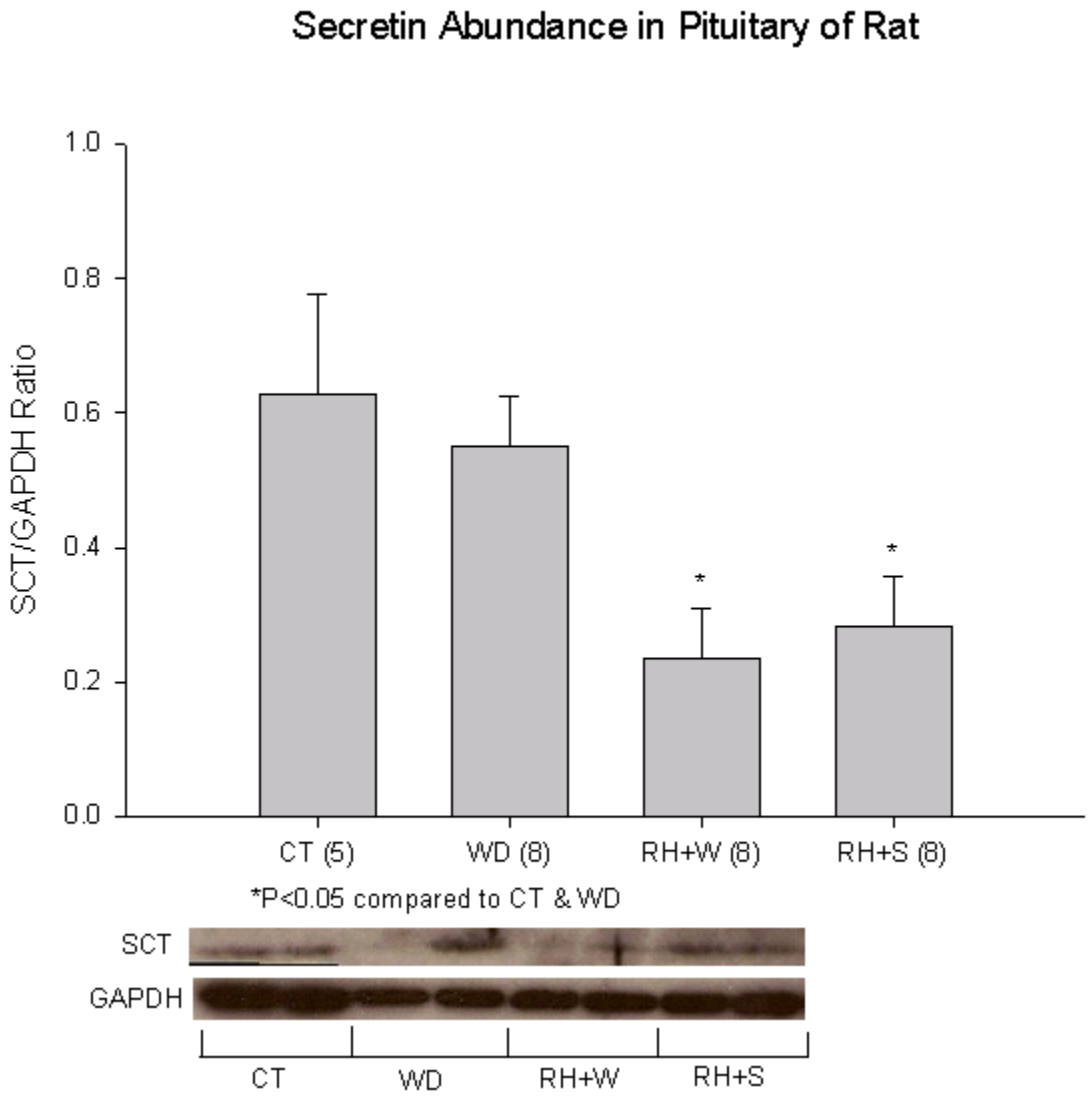

Secretin Abundance in Pituitary of Rat
SCT/GAPDH Ratio
1.0
0.8
0.6
0.4
0.2
0.0
*
*
CT (5)
WD (8)
RH+W (8)
RH+S (8)
*P<0.05 compared to CT & WD
SCT
GAPDH
CT
WD
RH+W
RH+S

Figure 2

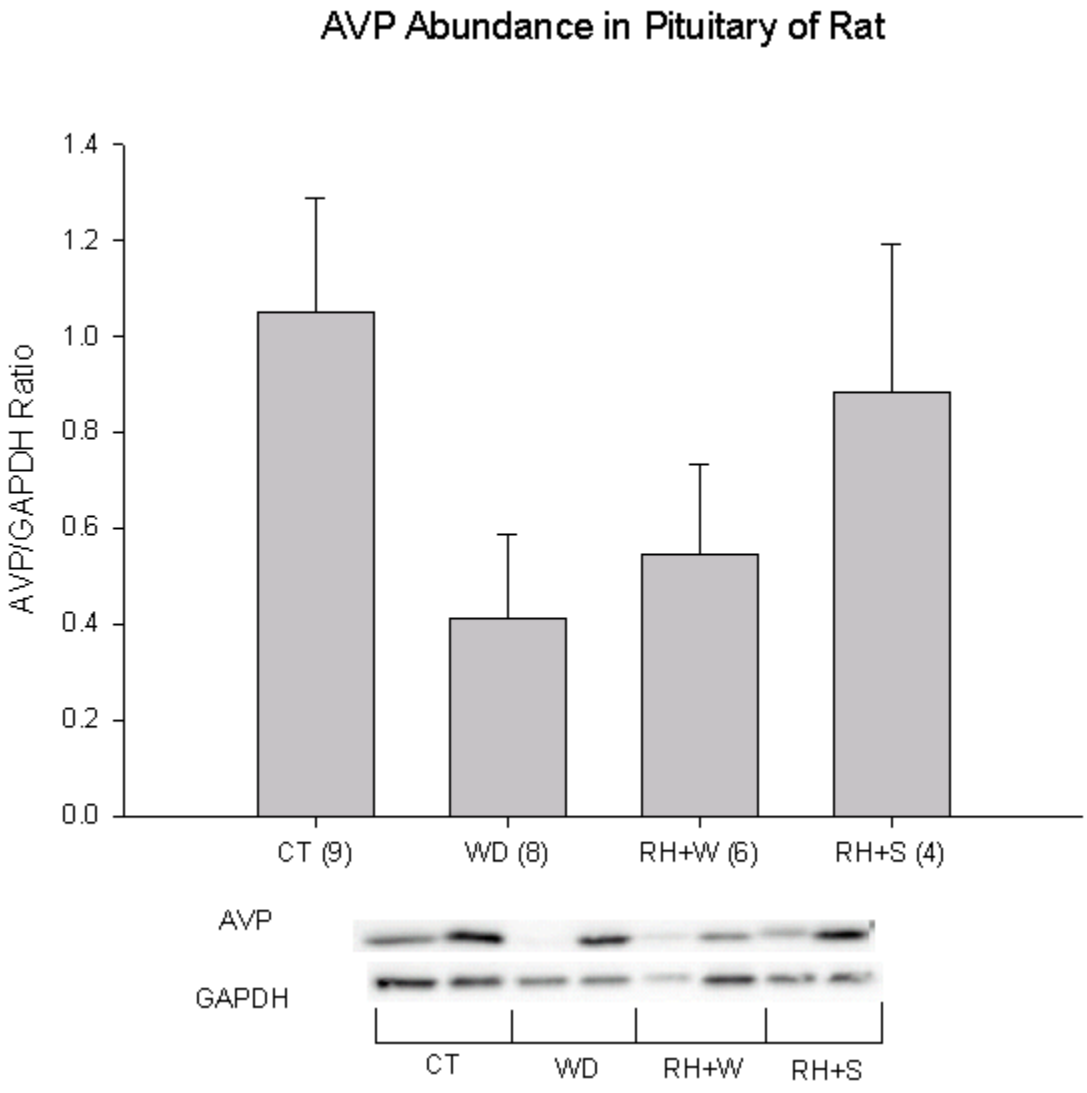

AVP Abundance in Pituitary of Rat
AVP/GAPDH Ratio
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CT (9)
WD (8)
RH+W (6)
RH+S (4)
AVP
GAPDH
CT
WD
RH+W
RH+S

Figure 3

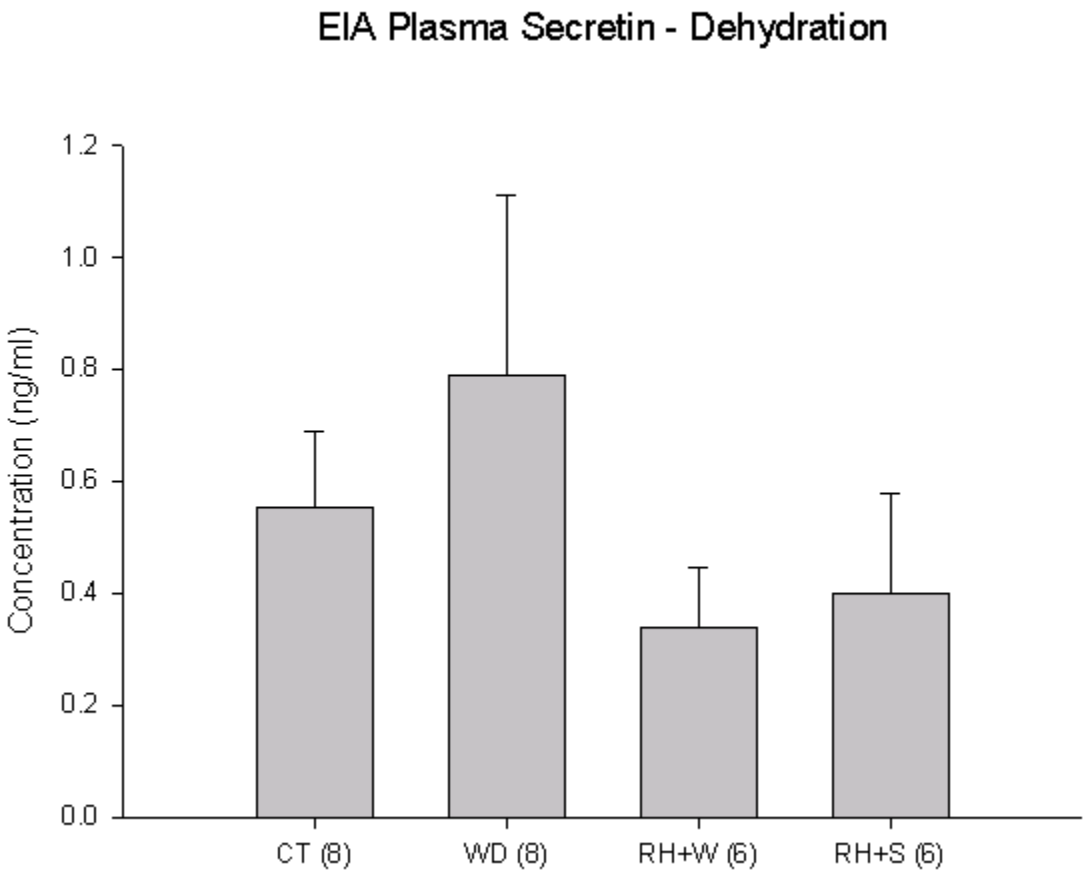

EIA Plasma Secretin - Dehydration
Concentration (ng/ml)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CT (8)
WD (8)
RH+W (6)
RH+S (6)

Figure 4

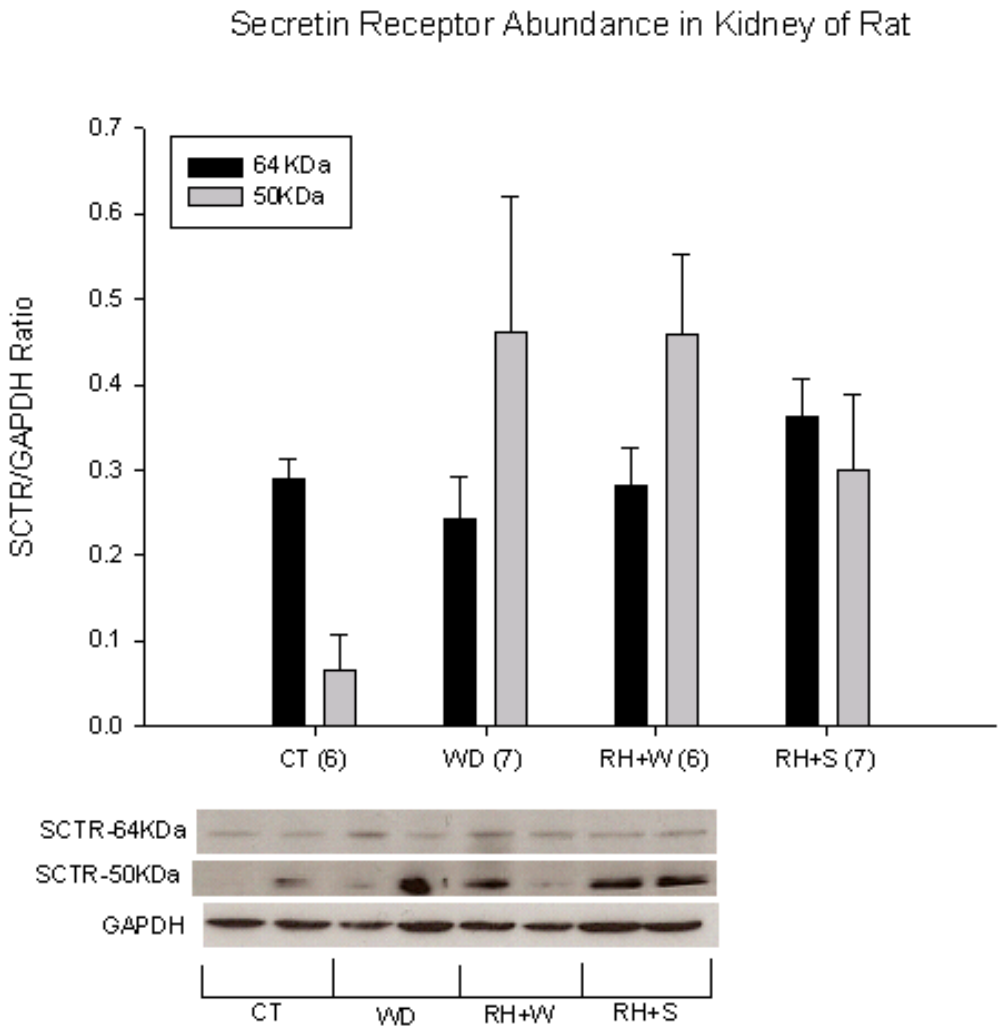

Secretin Receptor Abundance in Kidney of Rat
64 KDa
50KDa
SCTR/GAPDH Ratio
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CT (6)
WD (7)
RH+W (6)
RH+S (7)
SCTR-64KDa
SCTR-50KDa
GAPDH
CT
WD
RH+W
RH+S

Figure 5

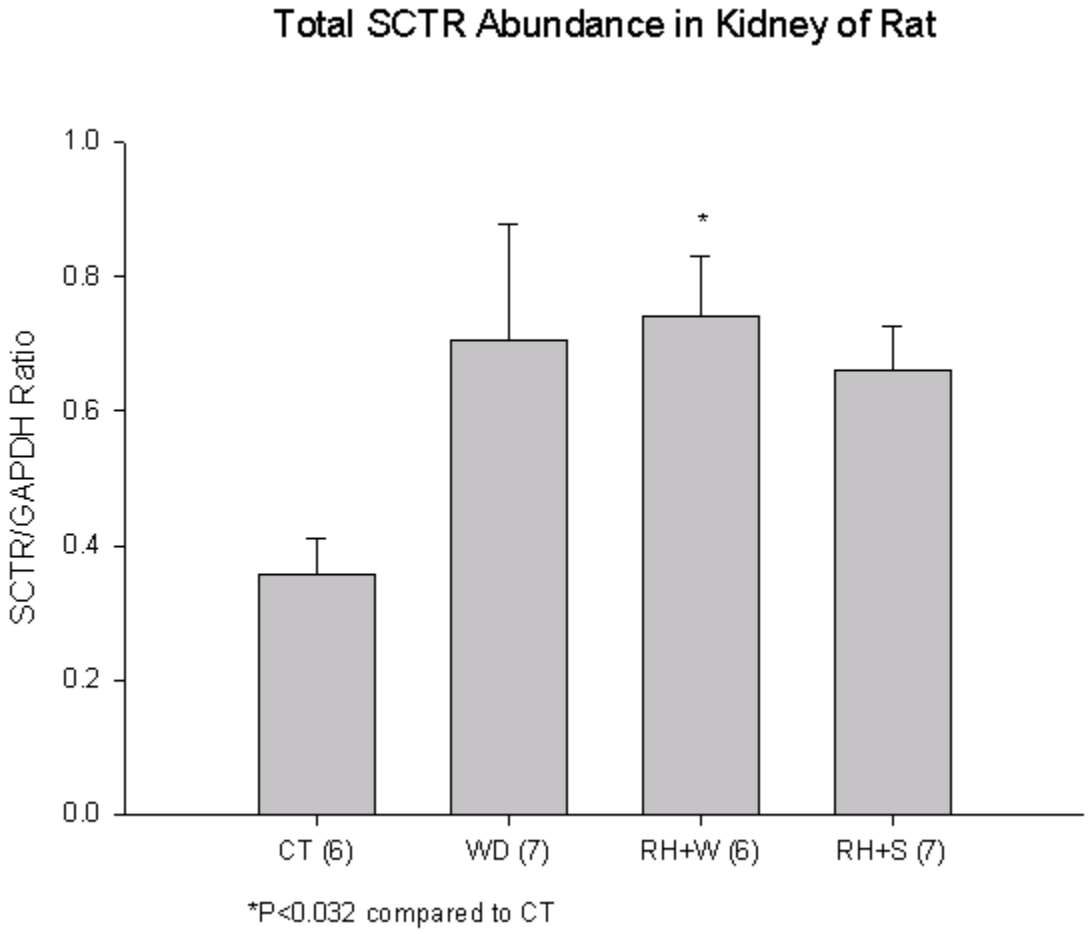

Total SCTR Abundance in Kidney of Rat
SCTR/GAPDH Ratio
1.0
0.8
0.6
0.4
0.2
0.0
*
CT (6)
WD (7)
RH+W (6)
RH+S (7)
*P<0.032 compared to CT

Figure 6

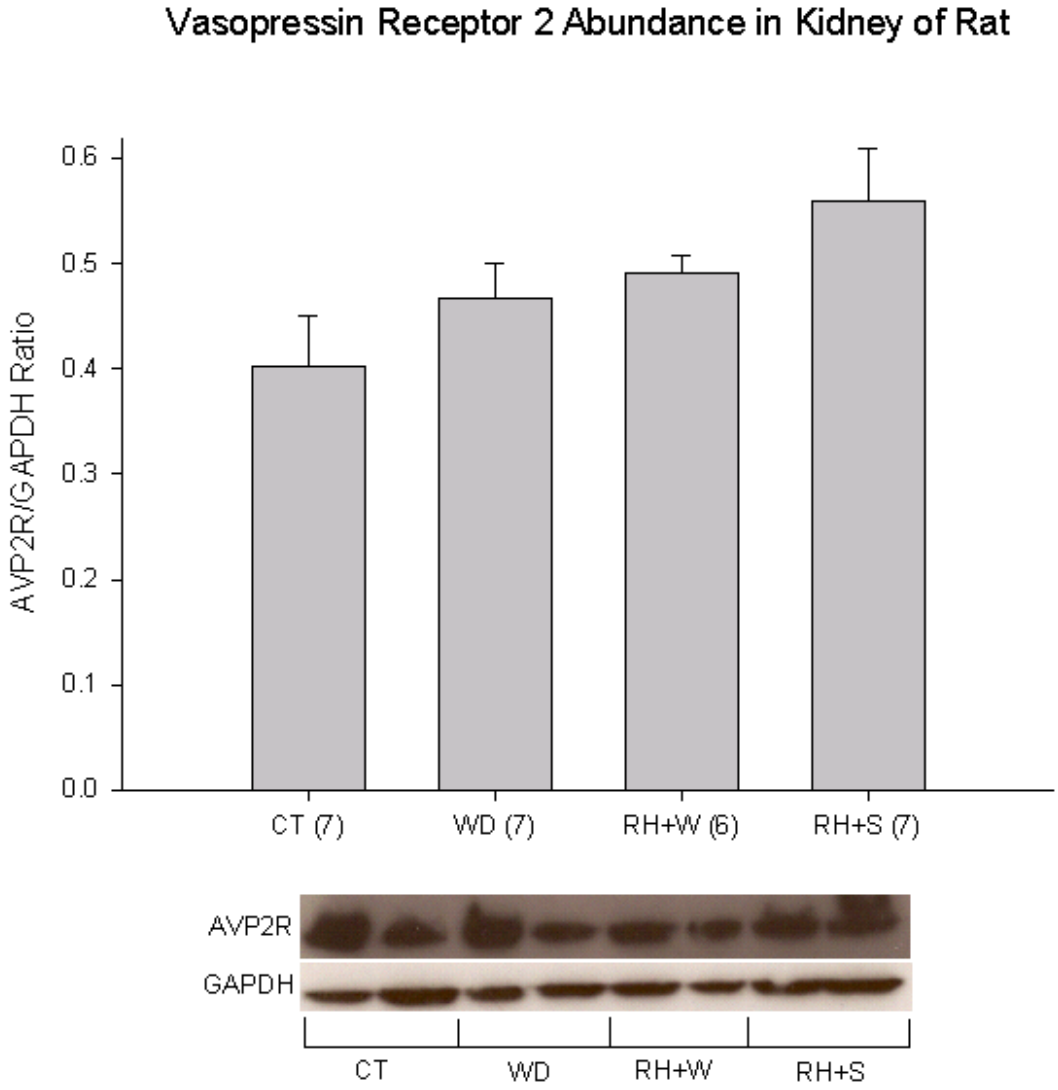

Vasopressin Receptor 2 Abundance in Kidney of Rat
AVP2R/GAPDH Ratio
0.0
0.1
0.2
0.3
0.4
0.5
0.6
CT (7)
WD (7)
RH+W (6)
RH+S (7)
AVP2R
GAPDH
CT
WD
RH+W
RH+S

Figure 7

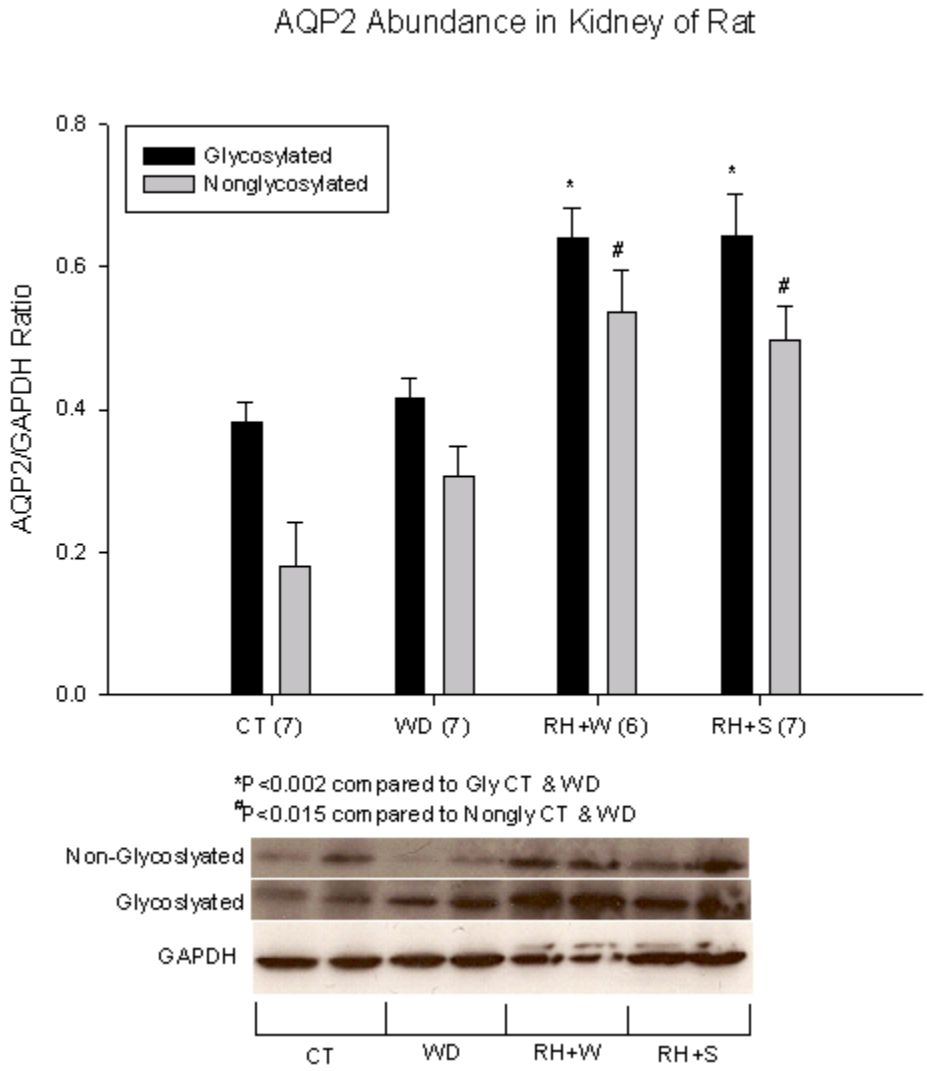

AQP2 Abundance in Kidney of Rat
Glycosylated
Nonglycosylated
AQP2/GAPDH Ratio
0.8
0.6
0.4
0.2
0.0
*
#
*
#
CT (7)
WD (7)
RH+W (6)
RH+S (7)
*P <0.002 compared to Gly CT & WD
#P<0.015 compared to Nongly CT & WD
Non-Glycosylated
Glycosylated
GAPDH
CT
WD
RH+W
RH+S

Figure 8

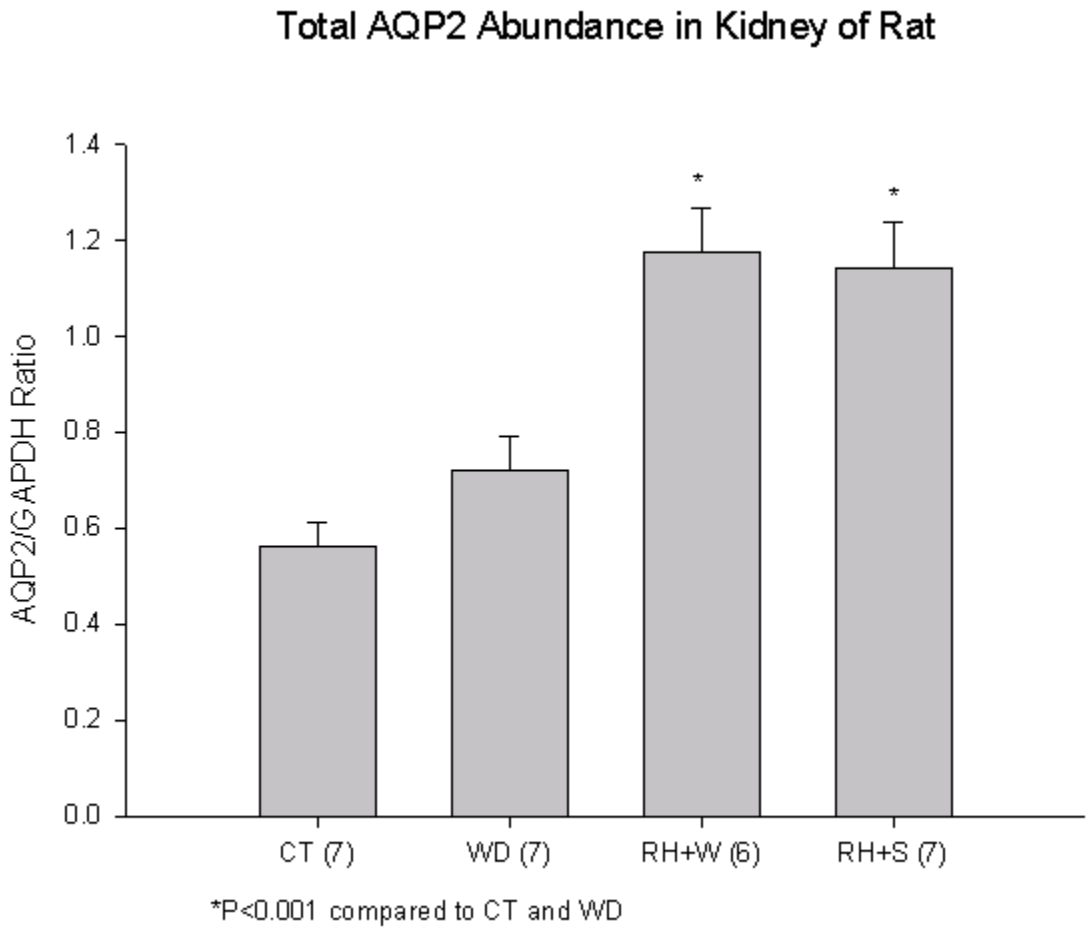

Total AQP2 Abundance in Kidney of Rat
AQP2/GAPDH Ratio
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
CT (7)
WD (7)
RH+W (6)
RH+S (7)
*P<0.001 compared to CT and WD

Figure 9

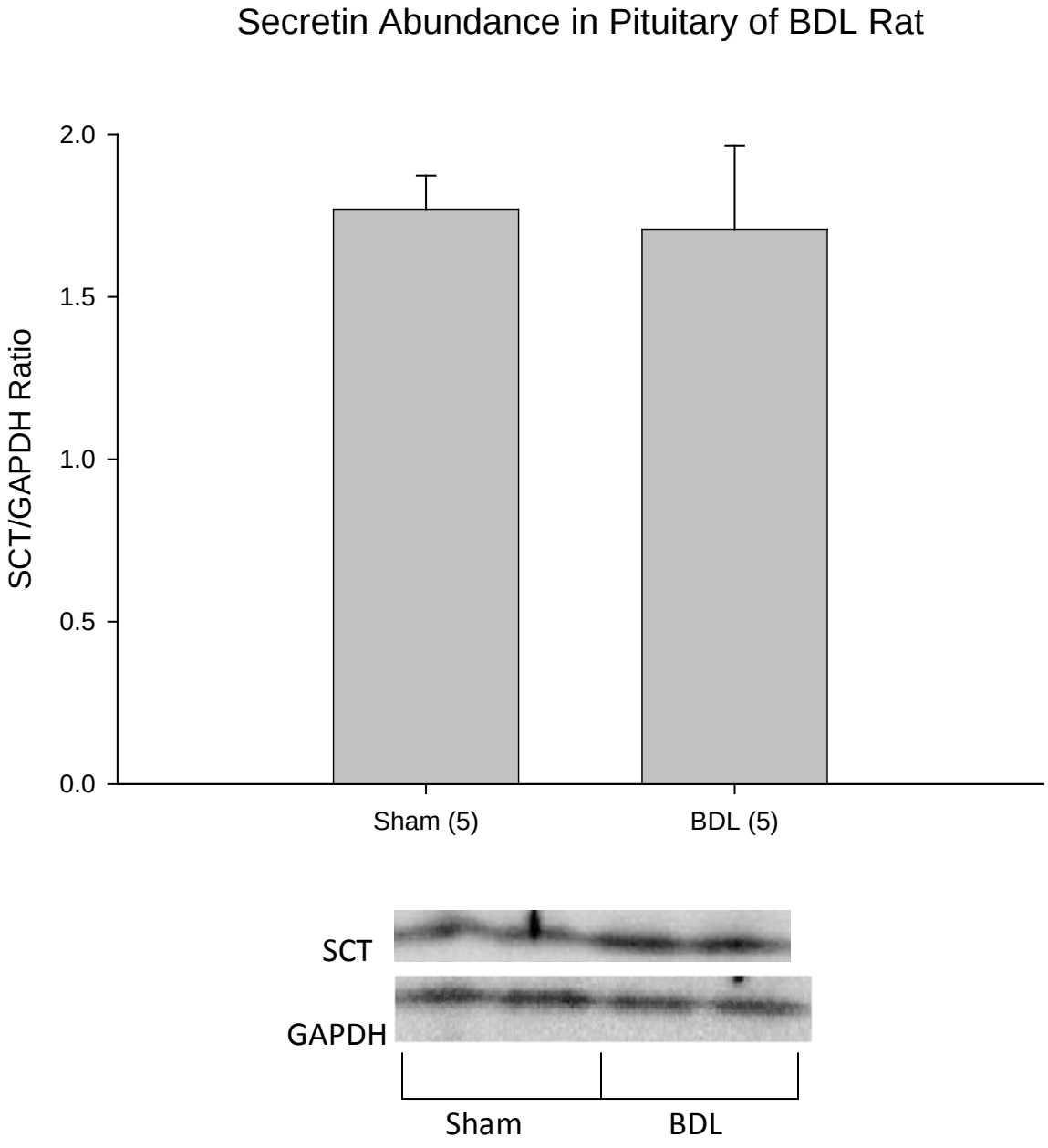

Secretin Abundance in Pituitary of BDL Rat
SCT/GAPDH Ratio
2.0
1.5
1.0
0.5
0.0
Sham (5)
BDL (5)
SCT
GAPDH
Sham
BDL

Figure 10

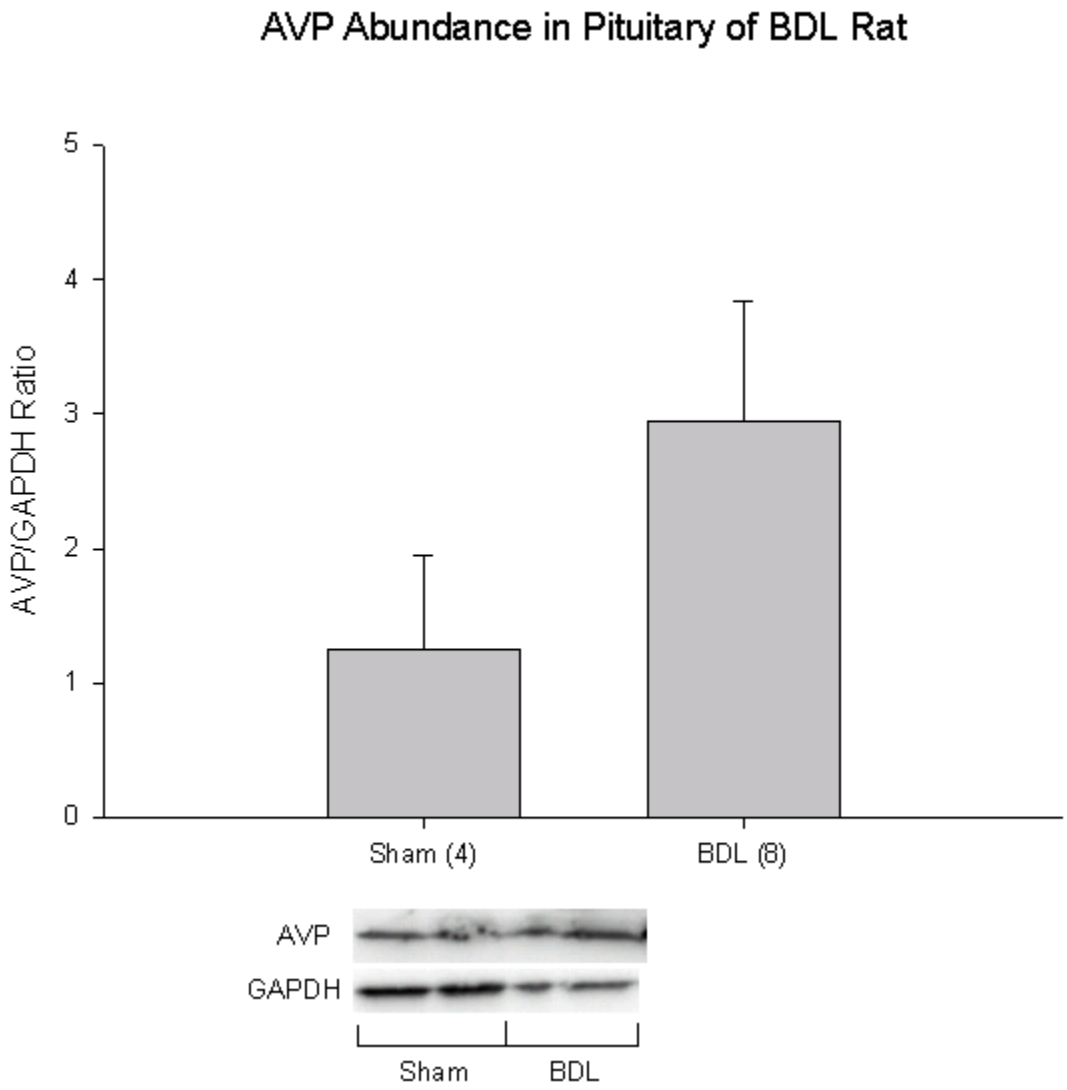

AVP Abundance in Pituitary of BDL Rat
AVP/GAPDH Ratio
5
4
3
2
1
0
Sham (4)
BDL (8)
AVP
GAPDH
Sham
BDL

Figure 11

Secretin Receptor Abundance at 64KDa in Kidney of BDL Rat

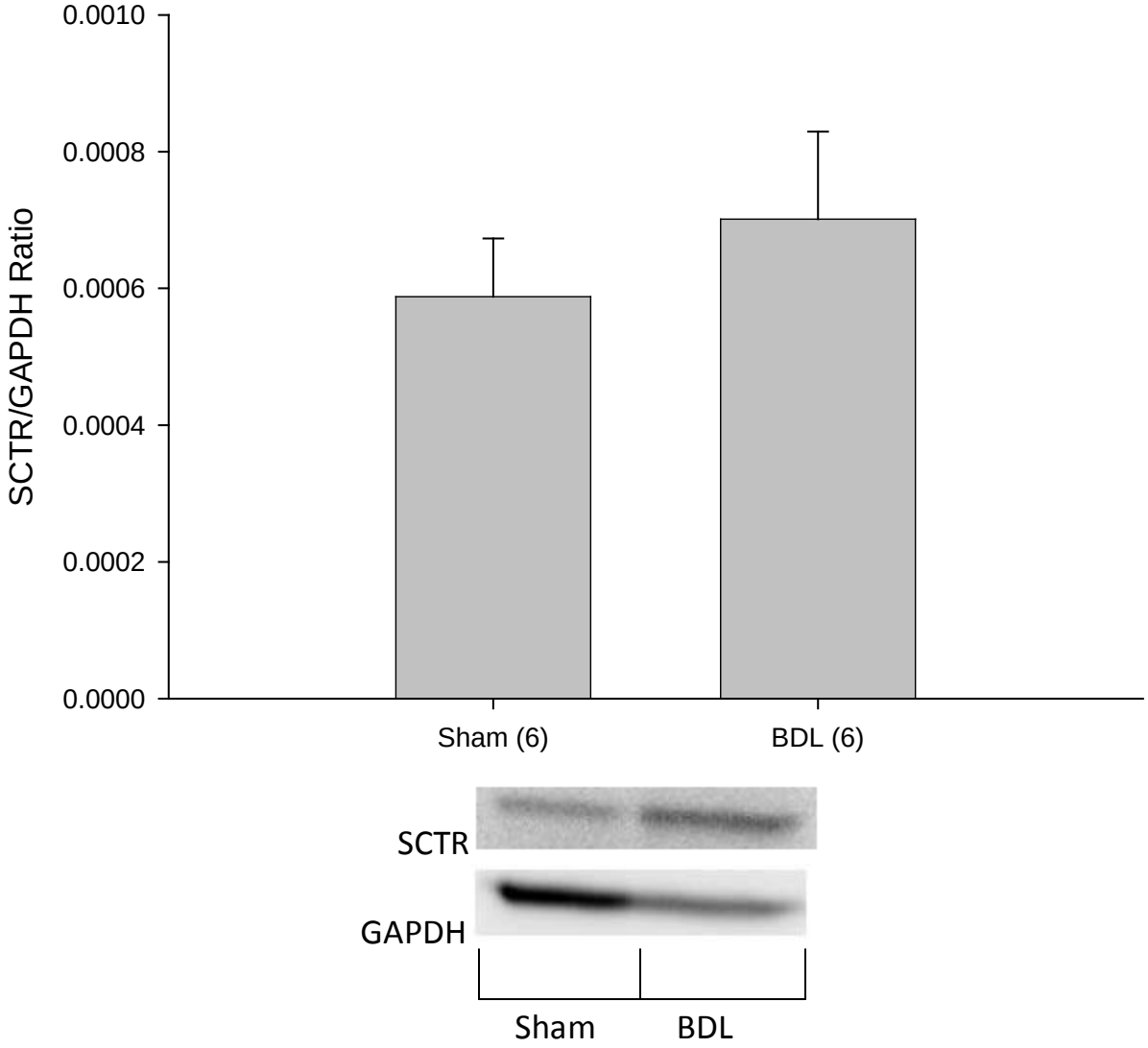
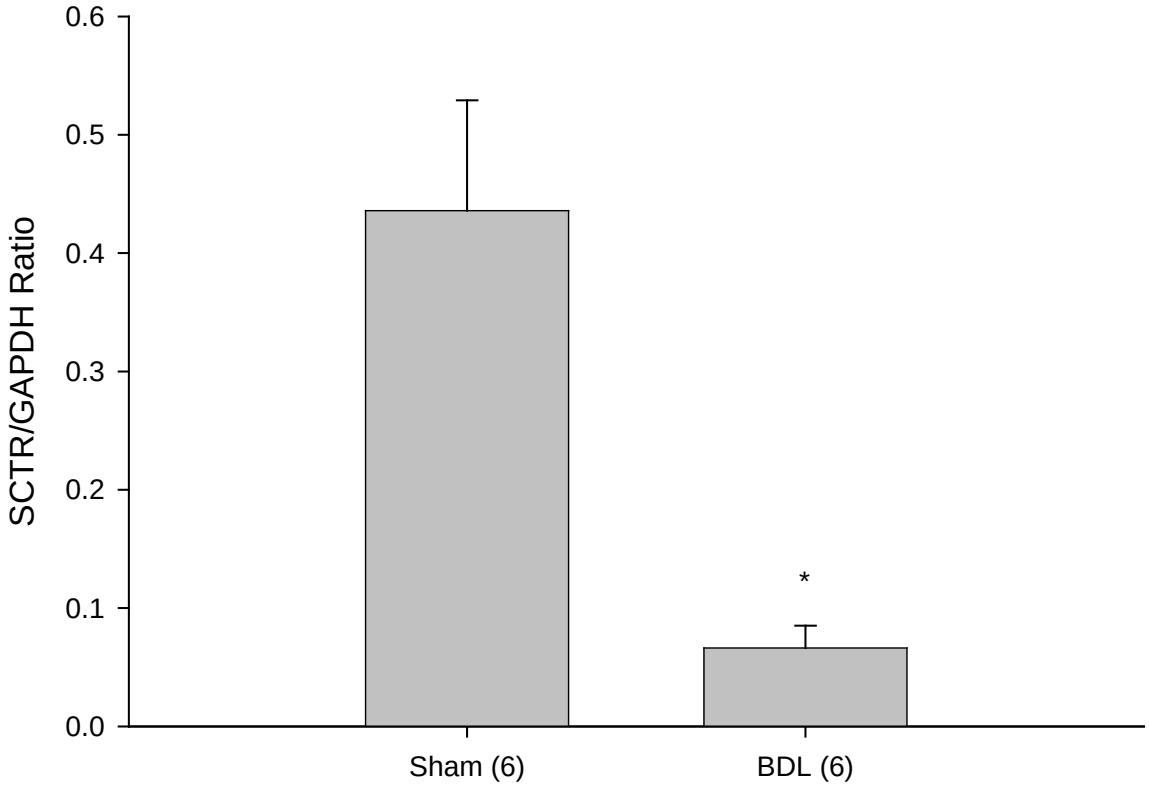


Figure 12

Secretin Receptor Abundance at 50KDa in Kidney of BDL Rat



*P<0.004 compared to Sham

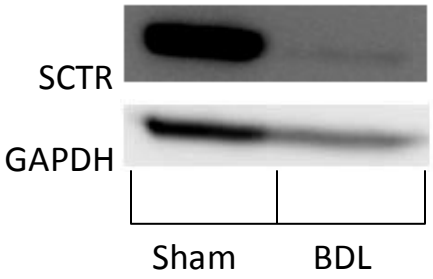


Figure 13

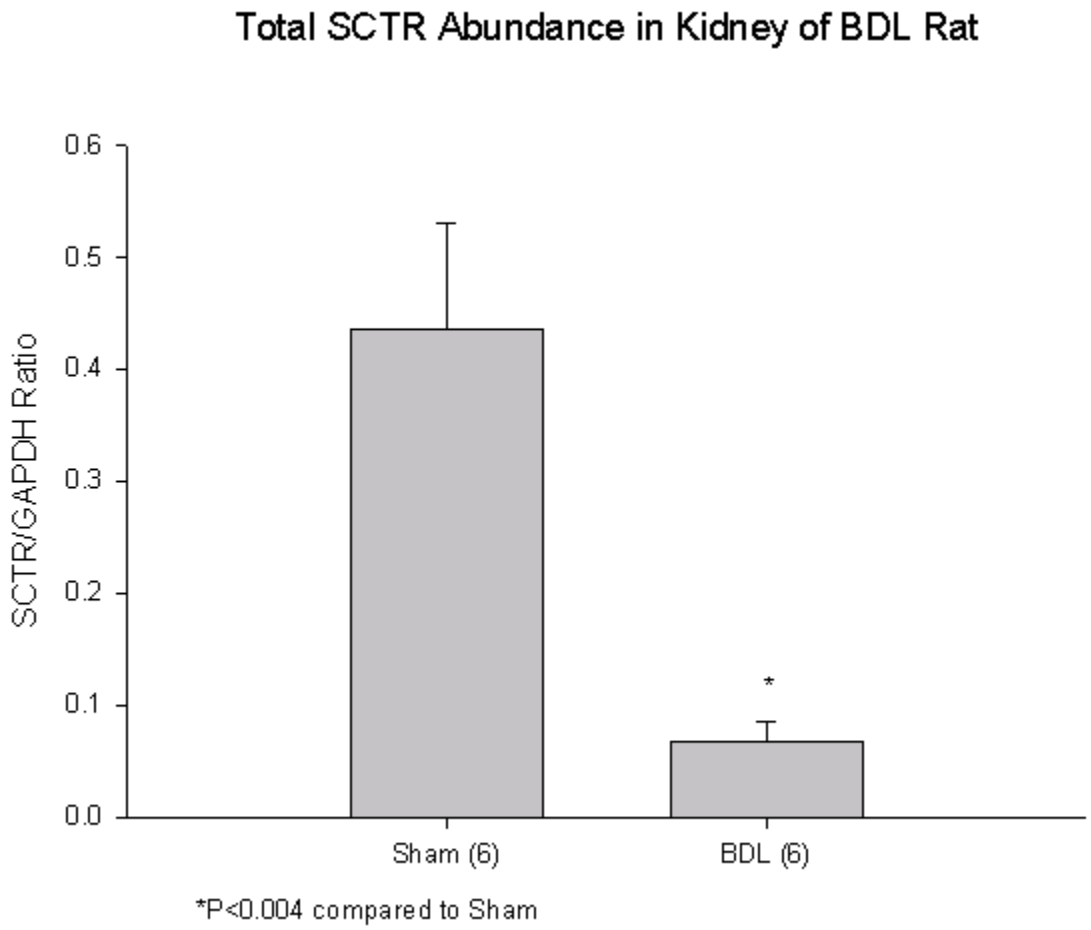
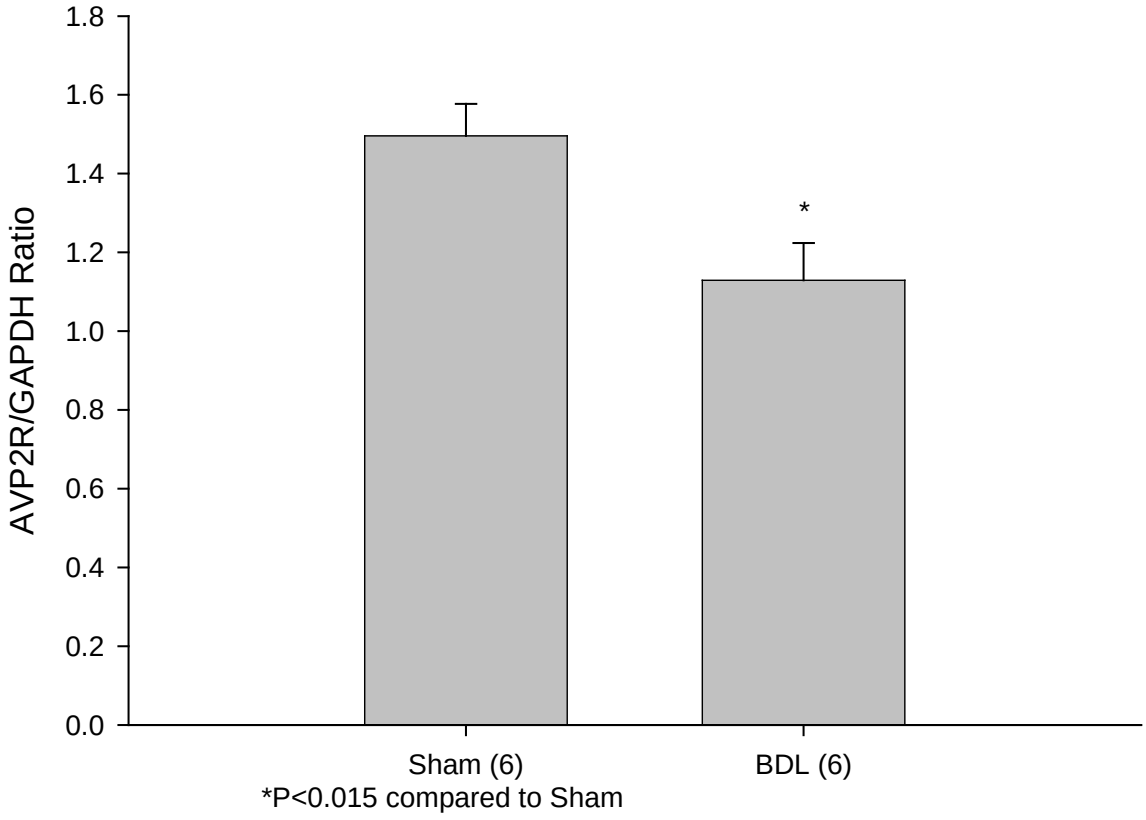

Total SCTR Abundance in Kidney of BDL Rat
SCTR/GAPDH Ratio
0.6
0.5
0.4
0.3
0.2
0.1
0.0
*
Sham (6)
BDL (6)
*P<0.004 compared to Sham

Figure 14

Vasopressin Receptor 2 Abundance in Kidney of BDL Rat



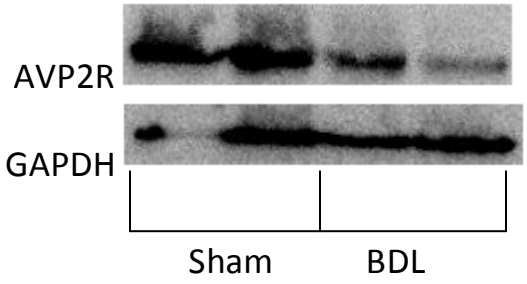


Figure 15

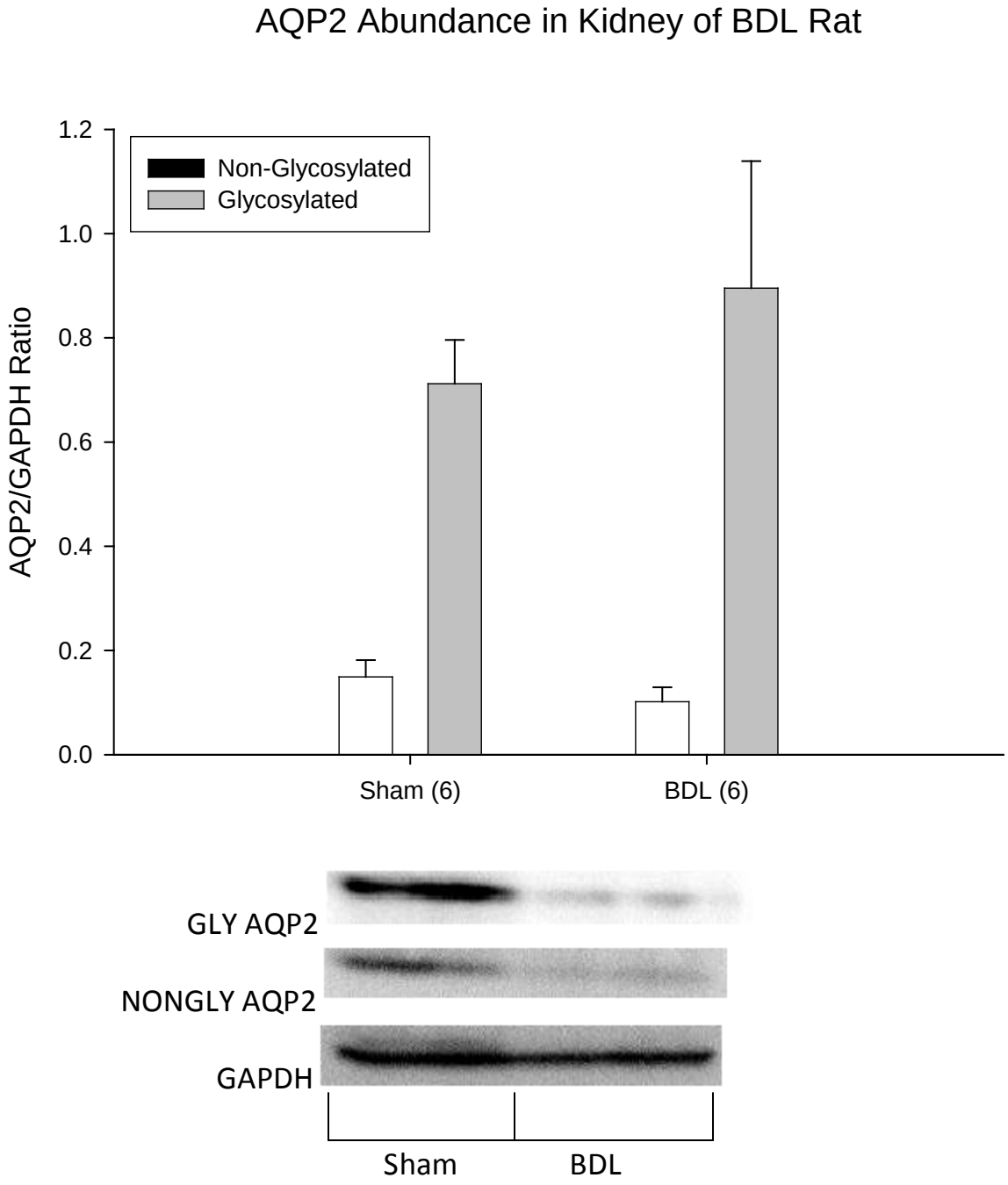

AQP2 Abundance in Kidney of BDL Rat
AQP2/GAPDH Ratio
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Non-Glycosylated
Glycosylated
Sham (6)
BDL (6)
GLY AQP2
NONGLY AQP2
GAPDH
Sham
BDL

Figure 16

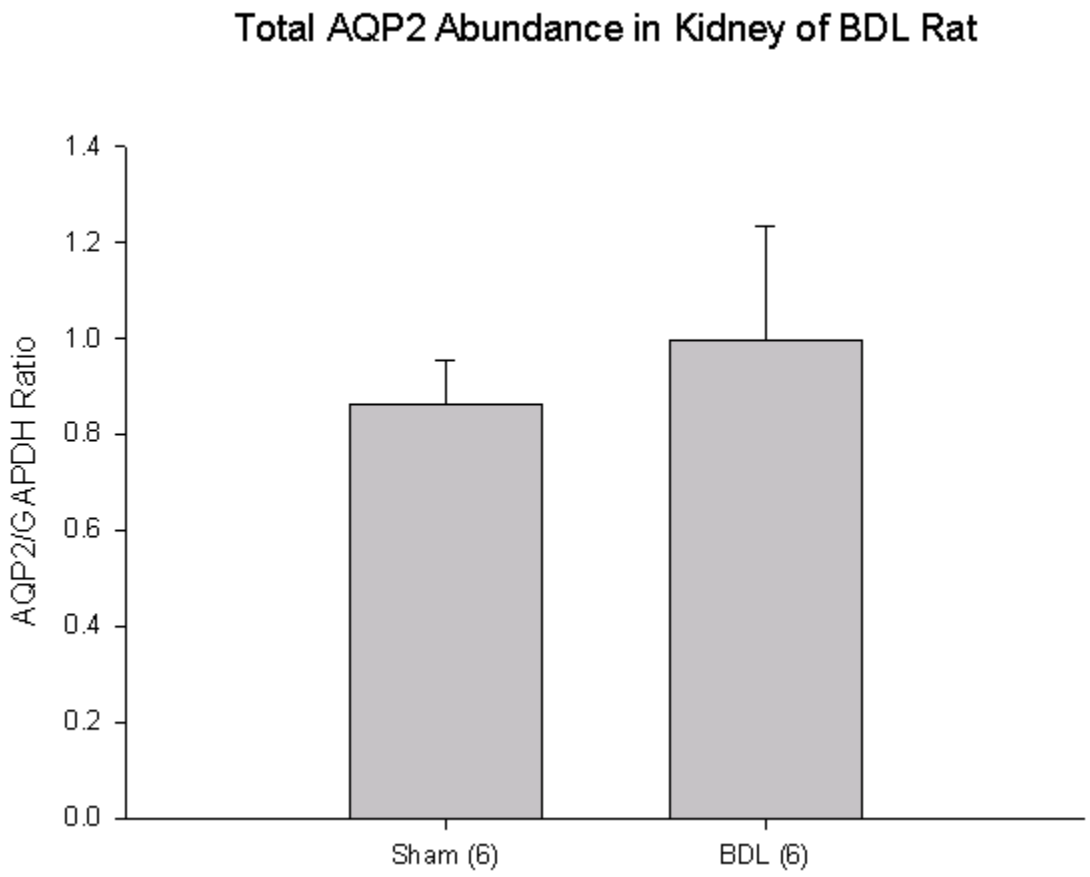

Total AQP2 Abundance in Kidney of BDL Rat
AQP2/GAPDH Ratio
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Sham (6)
BDL (6)

Figure 17

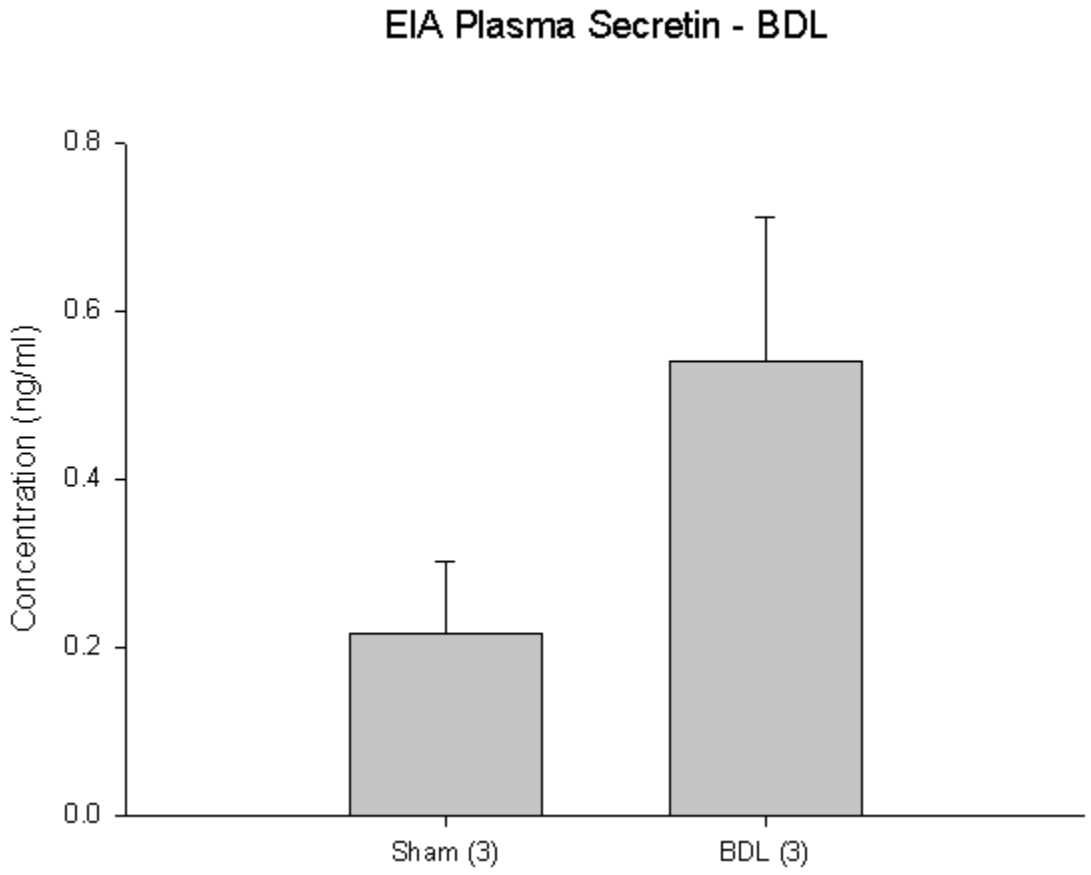

EIA Plasma Secretin - BDL
Concentration (ng/ml)
0.8
0.6
0.4
0.2
0.0
Sham (3)
BDL (3)

Diagram 2

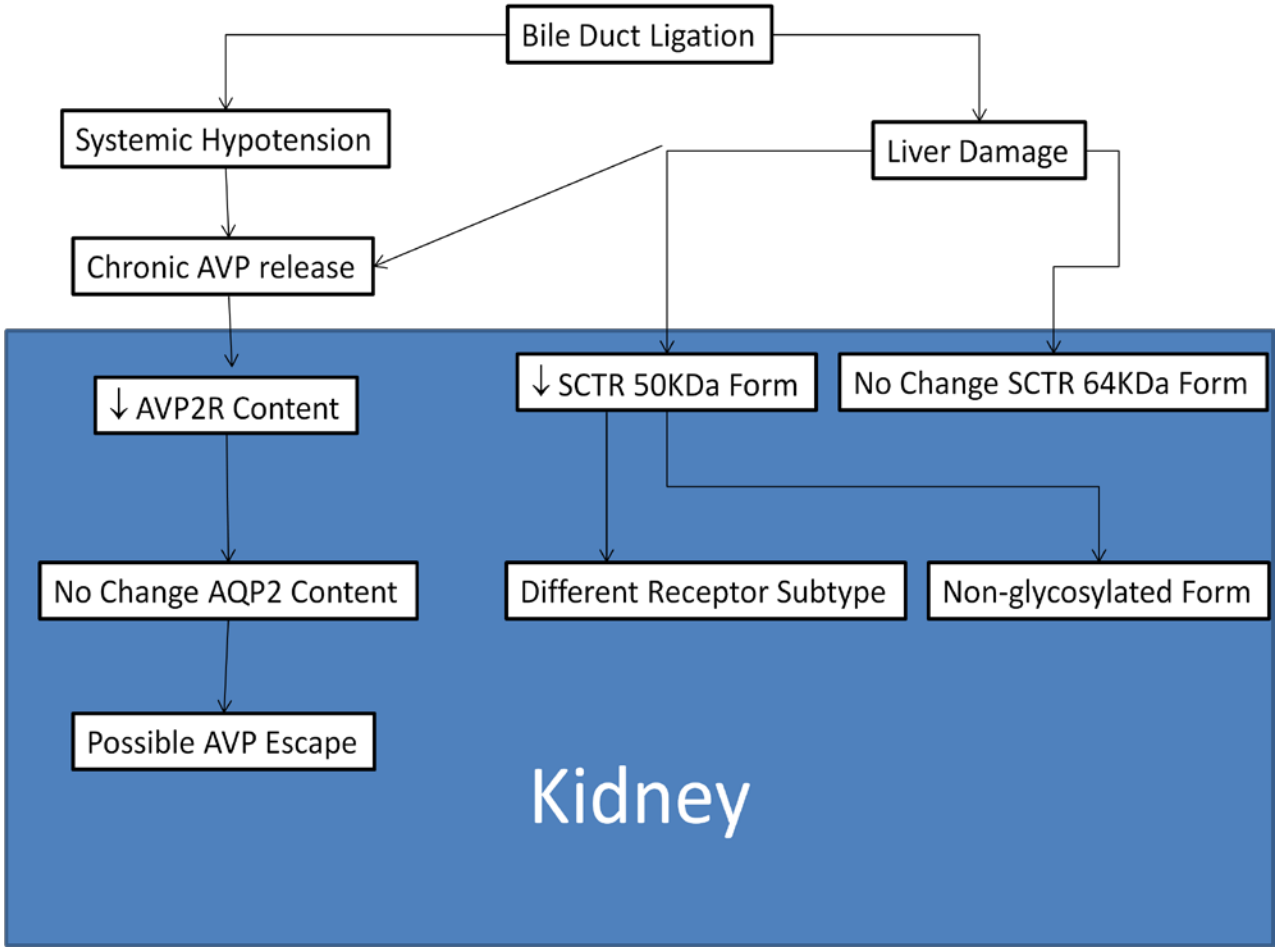

Bile Duct Ligation
Systemic Hypotension
Liver Damage
Chronic AVP release
↓ AVP2R Content
↓ SCTR 50KDa Form
No Change SCTR 64KDa Form
No Change AQP2 Content
Different Receptor Subtype
Non-glycosylated Form
Possible AVP Escape
Kidney

Figure Legend:

Diagram 1: Diagram 1 illustrates the physiology of water balance regulation. Increased plasma osmolality or decreased blood volume signals the brain and the pituitary to release vasopressin (AVP) and possibly secretin (SCT). AVP and SCT bind to their receptors, the vasopressin type-2 receptor (AVP2R) and secretin receptor (SCTR) respectively, in the kidney causing a rise in cAMP. Aquaporin 2 (AQP-2) channels undergo translocation from intracellular vesicles (IV) to the plasma membrane (PM) and allow for water retention. Water retention decreases osmolality and increases blood volume, which inhibits stimuli for AVP and SCT release from the pituitary.

Figure 1: Secretin (SCT) in the pituitary following 48hWD and 2h rehydration. Digital images of representative western blot analysis for SCT and GAPDH from pituitary of control (CT), 48hWD rats (WD), and rats given access to either water (RH+W) or saline (RH+S) for 2h after WD. Graph summarizes the densitometric analysis of SCT in the same groups. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. * Statistically significant, $P<0.05$. The number in parentheses indicates the number of animals in the respective group.

Figure 2: Arginine vasopressin (AVP) in the pituitary following 48hWD and 2h rehydration. Digital images of representative western blot analysis for AVP and GAPDH from pituitary of control (CT), 48hWD rats (WD), and rats given access to either water (RH+W) or saline (RH+S) for 2h after WD. Graph represents densitometric analysis of

AVP in control, 48hWD rats, and rats given access to water or saline for 2h after WD. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 3: SCT in the plasma following 48hWD and 2h rehydration. Graph represents the average plasma secretin concentration from controls (CT), 48hWD rats (WD), and rats given access to either water (RH+W) or saline (RH+S) for 2h after WD. The number in parentheses indicates the number of animals in the respective group.

Figure 4: Western blot analysis of secretin receptor (SCTR) in the kidney following 48hWD and 2h rehydration. Digital images of representative western blot analysis for SCTR at 64 and 50 kDa and GAPDH from kidney controls (CT), 48hWD rats (WD), and rats given access to either water (RH+W) or saline (RH+S) for 2h after WD. Graph represents densitometric analysis of the 64kDa band (black bars) and the 50kDa band (grey bars) of SCTR in the same groups. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 5: Densitometric analysis of the total Secretin Receptor (SCTR) in the kidney following 48hWD and 2h rehydration. Total SCTR was determined by adding the 50kDa and 64kDa bands (Figure 4) together. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. * Statistically

significant, P<0.032. The number in parentheses indicates the number of animals in the respective group.

Figure 6: Vasopressin 2 Receptor (AVP2R) in the kidney following 48hWD and 2h rehydration. Digital images of representative western blot analysis for AVP2R and GAPDH from kidney of controls (CT), 48hWD rats (WD), and rats given access to either water (RH+W) or saline (RH+S) for 2h after WD. Graph summarizes the densitometric analysis of AVP2R in the same groups. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 7: Western blot analysis of Aquaporin 2 water channel (AQP2) in the kidney following 48hWD and 2h rehydration. Digital images of a representative western blot for glycoslyated AQP2 (black bars), non-glycoslyated AQP2 (grey bars), and GAPDH from kidney of controls (CT), 48hWD rats (WD), and rats given access to either water (RH+W) or saline (RH+S) for 2h after WD. Graph summarizes the separate densitometric analysis of glycoslyated and non-glycoslyated AQP2 in the same groups. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. $^{*,\#}$ is statistically significant at P<0.002 & P<0.015 respectively. The number in parentheses indicates the number of animals in the respective group.

Figure 8: Densitometric analysis of total Aquaporin 2 water channel (AQP2) in the kidney following 48hWD and 2h rehydration shown in Figure 7. Bands for glycosylation and nonglycosylation were added together. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. *, Statistically significant, P<0.001. The number in parentheses indicates the number of animals in the respective group.

Figure 9: Secretin (SCT) in the pituitary following bile duct ligation (BDL) and sham ligation surgeries (Sham). Digital images of representative western blot for SCT and GAPDH from pituitary of sham and BDL rats. Graph represents densitometric analysis of SCT in sham and BDL rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 10: Vasopressin **(**AVP) in the pituitary following BDL and sham surgeries. Digital images of representative western blot analysis for AVP and GAPDH from pituitary of sham and BDL rats. Graph represents densitometric analysis of AVP in sham and BDL rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 11: Western blot analysis of 64kDa Secretin Receptor (SCTR) in the kidney following BDL and sham surgeries. Digital images of representative western blot analysis

for SCTR and GAPDH from kidney of BDL and sham rats. Graph represents densitometric analysis of SCTR in BDL and sham rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 12: Western blot analysis of 50kDa Secretin Receptor (SCTR) in the kidney following BDL and sham surgeries. Digital images of representative western blot analysis for SCTR and GAPDH from kidney of BDL and sham rats. Graph represents densitometric analysis of SCTR in BDL and sham rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. * Statistically significant, P<0.004. The number in parentheses indicates the number of animals in the respective group.

Figure 13: Total Secretin Receptor (SCTR) in the kidney following BDL and sham surgeries. Bands at 50KDa and 64KDa were added together. Digital images of representative western blot analysis for SCTR and GAPDH from kidney of BDL and sham rats. Graph represents densitometric analysis of SCTR in BDL and sham rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. * Statistically significant, P<0.004. The number in parentheses indicates the number of animals in the respective group.

Figure 14: Vasopressin 2 receptor (AVP2R) in the kidney following BDL and sham surgeries. Digital images of representative western blot analysis for AVP2R and GAPDH

from kidney of BDL and sham rats. Graph represents densitometric analysis of AVP2R in BDL and sham rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. * Statistically significant, P<0.015. The number in parentheses indicates the number of animals in the respective group.

Figure 15: Aquaporin 2 water channel (AQP2) in the kidney following BDL and sham surgeries. Digital images of representative western blot analysis for glycoslyated AQP2, non-glycoslyated AQP2, and GAPDH from kidney of BDL and sham rats. Graph represents densitometric analysis of glycoslyated (grey bars) and non-glycoslyated (black bars) AQP2 in BDL and sham rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 16: Densitometric analysis of total Aquaporin 2 water channel (AQP2) in the kidney following BDL and sham surgeries. Bands for glycosylation and nonglycosylation were added together. Digital images of representative western blot analysis for glycoslyated AQP2, non-glycoslyated AQP2, and GAPDH from kidney of BDL and sham rats. Graph represents densitometric analysis of glycoslyated and non-glycoslyated AQP2 in BDL and sham rats. Bands of interest were quantified by densitometry using Image J, and respective values were normalized by GAPDH. The number in parentheses indicates the number of animals in the respective group.

Figure 17: Secretin (SCT) in the plasma following BDL and sham surgeries. Graph represents the average plasma secretin concentration from BDL and sham rats. The number in parentheses indicates the number of animals in the respective group.

Diagram 2: Diagram of the possible effect of liver failure on renal function. Bile duct ligation (BDL) causes systemic hypotension and liver damage, which causes increased vasopressin (AVP) release. The vasopressin receptor (AVP2R) decreased and there was no change in aquaporin 2 (AQP-2) suggesting a possible AVP escape in these rats. Liver damage caused a decrease in the 50KDa form of the secretin receptor (SCTR) and no change in the 64KDa form suggesting there may either be two different receptor subtypes or that liver damage just affects the non-glycosylated form of the SCTR.