THAI JOURNAL OF PHYSIOLOGICAL SCIENCES
Volume 18 (No.2, August 2005)
Page 17-27
www.tjps.org
ISSN 0857 – 5754

Original Article

RENAL RESPONSE TO MESENTERIC INFUSION OF HYPERTONIC SALINE IN ADRIAMYCIN-TREATED RATS

Dusit Jirakulsomchok[1], N. Kunbootsri[1], S. Napawachirahat[1], Saowaluk Jirakulsomchok[2], J. Michael Wyss[3], and Sanya Roysommuti[1]

[1]Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand, [2]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand, [3]Department of Cell Biology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL35294-0019, USA

This study tested the hypothesis that loss of indirect hepatic regulation of the kidney contributes to sodium and water retention in rats in which the nephrotic syndrome was induced by a single intravenous injection of adriamycin. Two days after adriamycin treatment, the treated rats developed hypoalbuminemia and proteinuria. During the control period, the treated rats displayed urinary excretions of sodium (E_{Na}) and potassium (E_K) that were significantly lower than those of control rats. Animals were tested before and after continuous infusion of hypertonic saline at 20 μl/min for 30 min into either femoral vein or mesenteric vein. In the control rats, mesenteric (compared to femoral) vein infusion of hypertonic saline caused a more rapid and greater rise in E_{Na}. Mesenteric (but not femoral) vein infusions also increased glomerular filtration rate (GFR) and renal plasma flow (RPF) in the control rats. In contrast, in adriamycin-treated rats, neither route of administration caused a change in E_K or GFR. However, E_{Na}, RPF were slightly increased after hypertonic saline infusion into either vessel. These data suggest that impairment in hepatorenal reflex contributes to renal sodium retention in adriamycin-treated rats.

Key words: nephrotic syndrome, kidneys, adriamycin, hepatic nerve, hepatorenal reflex

Nephrotic syndrome is associated with salt and water retention by the kidney, and an associated edema that is the clinical manifestation of expanded interstitial volume (Schnaper and Robson, 2001). However, the mechanism underlying the retention of interstitial fluid has not been entirely understood. One potential mechanism is that the loss of albumin in the urine results in hypoalbuminemia and decreased plasma colloid osmotic pressure. The resulting hypovolemia could result in renal retention of excess salt and water (Schnaper and Robson, 2001). Alternatively, increased efferent renal nerve activity (DiBona et al., 1998; Herman et al., 1989; Koepke and DiBona, 1987), a blunted responses to atrial natriuretic peptide (ANP) (Koepke and DiBona, 1987; Morita et al., 1993) and a decrease in GFR might contribute to the fluid retention in this disease (Ichikawa et al., 1983; Koepke and DiBona, 1987; Schnaper and Robson, 2001).

The liver contains Na-sensitive mechanism that senses an increase in plasma sodium concentration and leads to natriuresis. Infusion of hypertonic NaCl into the splanchnic circulation results in a greater natriuresis than infusion into the inferior vena

Received: May 31, 2005 ; accepted: July 5, 2005
Correspondence should be addressed to Dr. Dusit Jirakulsomchok, Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
E-mail: jdusit@kku.ac.th

cava (Daly et al, 1967; Lopez-Novoa and Martinez-Maldonado, 1982; Strandhoy and Williamson, 1970). A large number of experiments suggest that mesenteric infusion of hypertonic NaCl stimulates the hepatoportal Na-sensitive mechanism. The signals travel via hepatic afferent nerves (Kobashi and Adachi, 1990; Morita et al., 1991b; Morita et al., 1997) to the central nervous system where they can decrease efferent renal sympathetic nerve activity, resulting in increased urinary Na excretion. This response is called hepatorenal reflex and it may play an important role in regulating body fluid balance (Morita et al., 1991; Morita et al., 1993). The hepatoportal NaCl receptors play a significant role in postprandial natriuresis (Morita et al., 1993). Intake of high NaCl food decreases renal nerve activity and increases urinary sodium excretion. This response is abolished by hepatic denervation (Morita et al., 1993). The pathological significance of this mechanism is demonstrated in the impairment of natriuretic responses to portal infusion of hypertonic saline in rats with liver cirrhosis (Lopez-Novoa and Martinez-Madonado, 1982). Interestingly, the hepatoportal NaCl sensitive mechanism has no significant effect on Na balance in cirrhotic animal fed normal NaCl food, but it plays an important role in Na balance when cirrhotic animals are fed high NaCl food (Morita et al., 1993).

A single i.v. injection of ADR was used to induce nephrotic syndrome in rats (DiBona et al., 1988; Hinojosa-Laborde et al., 1994; Koepke and DiBona, 1987; Perico et al, 1989). While abnormal sodium excretion has been studied in this model 2-3 weeks after adriamycin administration, the present study tested the hypothesis that the abnormal in sodium excretion is displayed early in adriamycin-induced nephrotic syndrome and that it may be related to an impaired response of hepatorenal reflex.

Materials and Methods

All studies were carried out under protocols reviewed and approved by the committee on Animal Research of the Faculty of Medicine, Khon Kaen University. Studies were performed in male Sprague-Dawley rats weighing 300-350 gm. Throughout the study all animals were allowed free access to water and standard laboratory rat chow and were housed in a constant temperature environment with a 12-hr light-dark cycle. Nephrotic syndrome was induced by a single intravenous injection of ADR (7.5 mg/kg bw), through the tail vein of unanesthetized animals. The development of the nephrotic syndrome was assessed by the appearance of protein in 24-hr urine samples. Control rats were injected with the same amount of isotonic saline vehicle. Two days after injection, 24-hr urinary protein excretion was measured via metabolic cage collections.

The experimental and control groups were further divided into two groups (8-10 animals in each group): Group 1. Control rats were infused with 5% NaCl, 20 μl/min for 30-min via the femoral vein (CFV); Group 2. Control rats were infused with 5% NaCl, 20 μl/min for 30-min via the mesenteric vein (CMV); Groups 3 and 4. Experimental rats were treated with adriamycin and infused with 5% NaCl, 20 μl/min for 30-min via the femoral (ADRFV; group 3) or mesenteric (ADRMV; group 4) vein.

On the experimental day, rats were anesthetized with pentobarbital sodium (60 mg/kg i.p.). A tracheotomy was performed for adequate ventilation. A catheter (PE 10 connected to PE 50) was placed in a femoral artery for measurement of arterial pressure and for collection of blood samples. The catheter was connected to a pressure transducer and continuous measurements of arterial pressure and heart rate using a recorder (Biopac system, DA 100 and MP 100, CA, USA). Mean arterial blood pressure (MAP) and heart rate (HR) were analyzed using Acknowledge software (version 3.5.5, Biopac). A catheter (PE 10) was placed either in the femoral or portal vein (via a branch of the mesenteric vein) for infusion of normal or hypertonic saline. Portal infusion was conducted via a needle connected to PE tubing inserted into a small branch of mesenteric vein pointed to the portal vein with no ligation of mesenteric vein, enabling the infusate to flow into

portal vein without severely disturbing mesenteric flow. The catheter was connected to an infusion pump (Harvard Apparatus, model 940, USA). A catheter (PE 50) was placed in a jugular vein and a blood sample (0.4 ml) was collected for analysis of plasma albumin and triglyceride (fully automate, Beckman series CX-7). The catheter was then connected to an infusion pump (Harvard model 940, USA) which delivered 0.9% NaCl at 100 μl/min for 50 min, thus providing adequate flow, and for infusion of inulin and para-aminohippuric acid (PAH). Then a priming dose of 0.5 ml of 5% inulin and 5% PAH in 0.9% NaCl (200 μl/min, with a sustaining flow of 20 μl/min was delivered throughout the experiments) was infused to measure inulin and PAH clearances. The ureters were cannulated (PE 10) and urine samples were collected separately into pre-weighed vials, and urine flow rates were calculated.

After a 60-min stabilization period, two consecutive 20-min control collections were followed by four consecutive 20-min experimental collections. A blood sample (0.3 ml) was collected in a heparinized syringe from the arterial catheter at the middle of each clearance period. The blood was centrifuged immediately, plasma was separated, and the packed red cells were resuspended with an equivalent volume of normal saline and replaced back to the animal. At the end of the experiment the animals were killed by an over dose of pentobarbital, and the kidneys were removed, blotted to dry and weighed.

Urine volume was determined gravimetrically, and urine sodium and potassium concentrations were determined by lithium internal standard flame photometry (Hitachi, model 775-A, Tokyo, Japan). The urine and plasma concentrations of inulin were determined by the anthrone method previously described by Davidson and Sacker (1963). Determination of paraaminohippuric acid (PAH) concentrations of urine and plasma were conducted by the method of Bratton and Marshall, as modified by Smith (Smith, 1956). Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated from the inulin and PAH clearance rates, respectively. Sodium and potassium excretion rates were calculated as the product of urine flow rate and urine sodium and potassium concentrations, respectively.

The data were expressed as means ± SEM. The values from two control clearance periods were averaged and compared to each of the experimental periods.

Statistical analyses

Statistical analyses of the data were performed using one-way ANOVA with post-hoc tests (Newman-Keuls), and data between groups were compared by the unpaired t-test. Statistical significance was defined as $p < 0.05$.

Table 1. Plasma albumin and urinary protein excretion (24 hr) in control and ADR rats

Parameters	Control (n=16)	ADR (n=16)
Plasma albumin, g/dl	2.75 ± 0.05	2.24 ± 0.14 *
Urine protein, mg /24 hr	7.13 ± 0.26	8.56 ± 0.58 *

Values are means ± SEM. Rats were kept in metabolic cage and urine was collected for 24 hrs for analysis of protein excretion. After anesthetized the rats, bloods were collected for analysis of plasma albumin. *, $p < 0.05$ control vs ADR; ADR, adriamycin-treated rats.

Table 2. Baseline data, control period after the stabilization period

Parameters	Control (n = 16)	ADR (n = 16)
MAP (mmHg)	111.9 ± 2.4	122.0 ± 2.5
HR (beat/min)	385 ± 13	364± 6
Hct (%)	47.05 ± 0.78	45.23 ± 0.40*
RPF (ml/min.gKW)	3.25 ± 0.13	2.94 ± 0.13
GFR (ml/min. gKW)	1.06 ± 0.07	1.10 ± 0.04
V (μl/min.gKW)	10.24 ± 0.83	9.45 ± 0.98
P_{Na} (meq/l)	144.47 ± 0.44	140.59 ± 1.59*
P_K (meq/l)	3.14 ± 0.06	2.92 ± 0.06*
E_{Na} (μEq/min.gKW)	2.48 ± 0.22	1.80 ± 0.14*
E_K (μEq/min.gKW)	1.55 ± 0.06	1.32 ±0.06*

Values are means ± SE; ADR, adriamycin treated rats; MAP, mean arterial pressure; HR, heart rate; Hct %, hematocrit; RPF, renal plasma flow; GFR, glomerular filtration rate; V, urine flow rate; P_{Na}, plasma sodium; P_K, plasma potassium; E_{Na}, sodium excretion; E_K, potassium excretion.

Results

Baseline data

As shown in Table 1, urinary protein excretion significantly increased (35 %; $p < 0.05$) in the rats that were infused with adriamycin, compared to control rats. Conversely, plasma albumin (19 %) was significantly decreased in adriamycin compared to control rats ($p < 0.05$). No ascites was observed in ADR rats. There were no differences in MAP, HR, RPF and GFR between the groups (Table 2); however, %hematocrit, plasma sodium and potassium concentrations and urinary Na excretion and K excretion were slightly decreased in adriamycin-treated compared to control rats ($p < 0.05$; Table 2).

Control rat responses to hypertonic saline infusion

In control rats the mesenteric infusion of hypertonic saline produced immediate increase in diuresis that was significantly greater than that displayed following saline infusion into the femoral vein (Fig. 1). The saline infusion into the mesenteric vein significantly increased diuresis for over 80 min, but the infusion into the femoral vein significantly increased diuresis for only 40 min. In control rats, infusion of hypertonic saline into either femoral vein or mesenteric vein increased Na excretion, but the infusion of hypertonic saline into mesenteric (compared to femoral) vein caused a more rapid response and larger magnitude response (Fig. 2, top). Hypertonic saline infusion into the mesenteric vein, increased Na excretion by 20-min post-infusion, but the femoral vein infusion

did not increase Na excretion until 40 min after infusion. The mesenteric (but not femoral) vein hypertonic saline infusion significantly increased potassium excretion ($p < 0.05$; Figure 3). No different changes in plasma Na or K concentrations before and after hypertonic saline infusion were observed (data not shown).

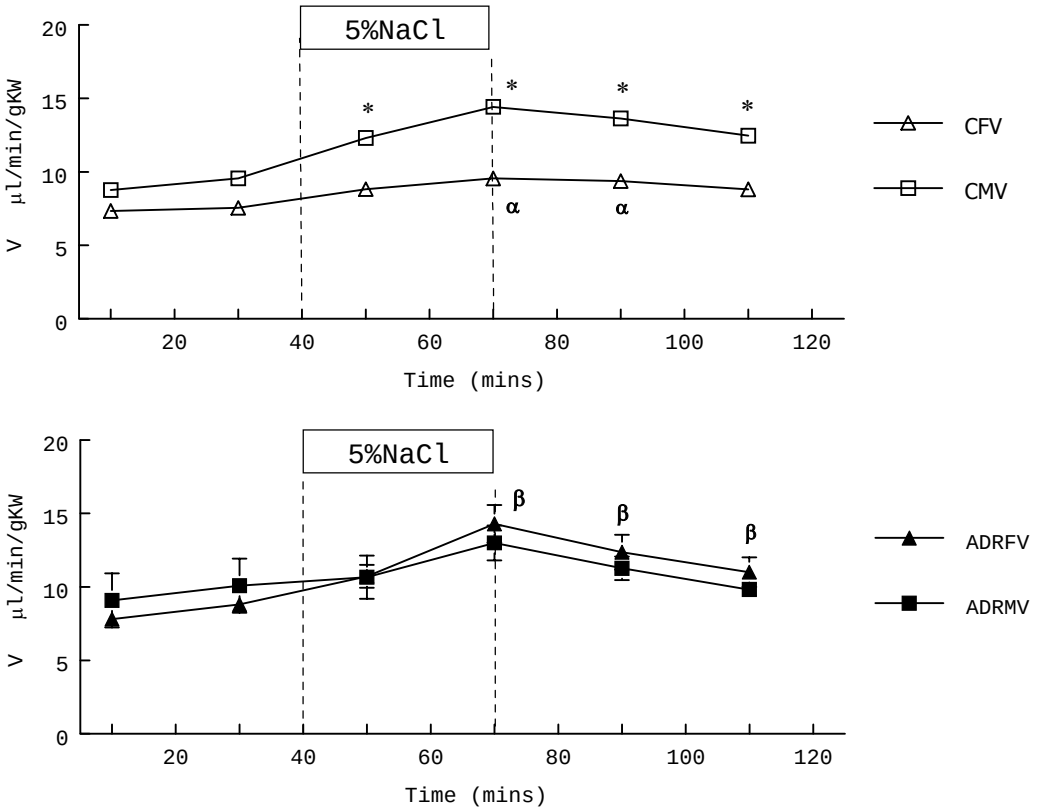

Figure 1. Urinary flow rate (V) before and after hypertonic saline infusion into either mesenteric vein (MV) or femoral vein (FV) of control and ADR rats. CFV, CMV = control rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; ADRFV, ADRMV = adriamycin treated rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; α, $p < 0.05$ compared with control period in CFV group; *, $p < 0.05$ compared with control period in CMV group; β, $p < 0.05$ compared with control period in ADRFV group; ψ, $p < 0.05$ compared with control period in ADRMV group; N = 8 –10 rats in every group.

Mesenteric infusion of hypertonic saline in control rats resulted in an increase in GFR and RPF ($p < 0.05$), but there were no significant changes in GFR and RPF in rats with infusion into femoral vein (Fig. 4, top and Fig.5, top).

There were no significant changes in MAP and HR between the two control groups during the experiments. MAP in femoral infusion was 98±10 mmHg in control period and 93±11 mmHg in the last infusion period. Infusion of hypertonic saline into mesenteric vein did not alter MAP (112±4 vs 105±5 mmHg).

Response to hypertonic saline infusion in the adriamycin-treated rats

The hypertonic saline infusion into either femoral vein or portal vein resulted in similar increases in diuresis (Fig. 1, bottom). The hypertonic saline infusion increased Na excretion similarly in the two adriamycin-treated groups, and in both groups the increases were similar to those observed in the control femoral infusion group, but much less than those observed in the control mesenteric group (Fig. 2). The hypertonic infusion caused no change in K excretion in either adriamycin-treated group (Fig. 3, bottom). Similarly, in the adriamycin-treated group, the infusions did not alter GFR (Fig. 4, bottom). However, in the adriamycin-treated rats infused with saline into either femoral vein or mesenteric vein showed a small and similar increase in RPF (Fig. 5, bottom). The infusion of hypertonic saline caused no significant alterations in MAP and HR in either adriamycin-treated group (data not shown).

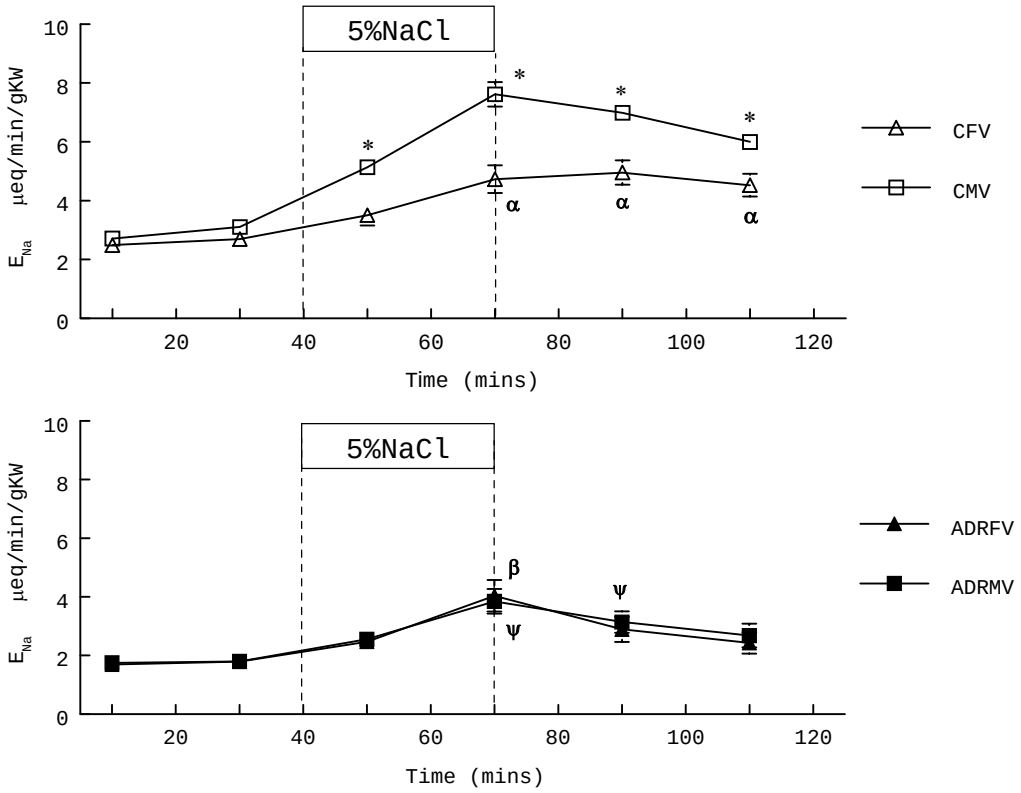

Figure 2. Urinary sodium excretion (E_{Na}) before and after hypertonic saline infusion into either mesenteric vein (MV) or femoral vein (FV) of control and ADR rats. CFV, CMV = control rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; ADRFV, ADRMV = adriamycin treated rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; α, $p < 0.05$ compared with control period in CFV group; *, $p < 0.05$ compared with control period in CMV group; β, $p < 0.05$ compared with control period in ADRFV group; ψ, $p < 0.05$ compared with control period in ADRMV group; N = 8 –10 rats in every group.

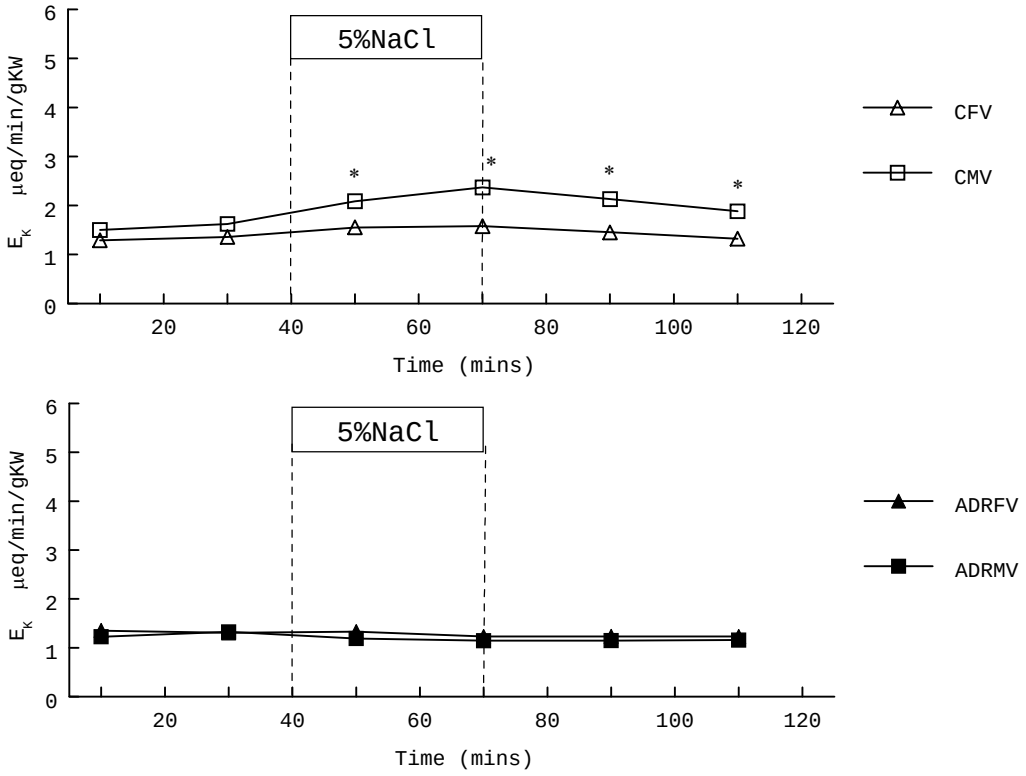

Figure 3. Urinary potassium excretion (E_K) before and after hypertonic saline infusion into either mesenteric vein (MV) or femoral vein (FV) of control and ADR rats. CFV, CMV = control rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; ADRFV, ADRMV = adriamycin treated rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; α, $p < 0.05$ compared with control period in CFV group; *, $p < 0.05$ compared with control period in CMV group; β, $p < 0.05$ compared with control period in ADRFV group; ψ, $p < 0.05$ compared with control period in ADRMV group; N = 8-10 rats in every group.

Discussion

Increased renal sodium and water retention contributes significantly to edema formation characteristic of nephrotic syndrome (NS), and increased efferent renal sympathetic nerve activity (ERSA) contributes to the excess retention (DiBona et al., 1988; DiBona and Kopp, 1997), Nephrotic rats display blunted response to intravenous isotonic saline volume loading (DiBona et al., 1988), largely due to attenuated inhibition of ERSNA during the period of volume loading (DiBona et al., 1988; DiBona and Kopp, 1997). Sodium receptors in the hepatic portal system play a role in regulating renal sodium excretion in response to changes in the concentration of Na^+ (Daly et al., 1967; Haberich, 1971; Lopez-Nova and Martinez-Maldonado, 1982; Morita et al., 1991b; Morita et al., 1993; Strandhoy and Williamson, 1970). The present data indicate that the hepatorenal reflex in control of sodium and water excretion is impaired in acute adriamycin-treated rats.

Several studies on experimental nephrotic syndrome have shown that sodium excretion is significantly impaired in nephrotic (compared to control) animals (DiBona et al., 1988; DiBona and Kopp, 1997; Herman et al., 1989; Koepke and DiBona, 1987). The present studies also demonstrate decreased sodium and potassium excretion in acute adriamycin-treated rats. Moreover, decreases in sodium and potassium excretion occur in the absence of changes in RPF and GFR, thus, the data

suggest increases in renal tubular reabsorption of sodium and potassium in adriamycin-treated group.

Control rats consistently displayed a more rapid natriuresis in response to hypertonic saline given into portal vein compared to femoral circulation. Maximal sodium excretion occurred during the initial 40 minutes after infusion and remained significantly elevated for > 100 min. These results support previous reports (Morita et al., 1993; Strandhoy and Williamson, 1970) and provide further evidence for the ability of the hepatic portal system to monitor plasma sodium concentration and thereby induce natriuresis.

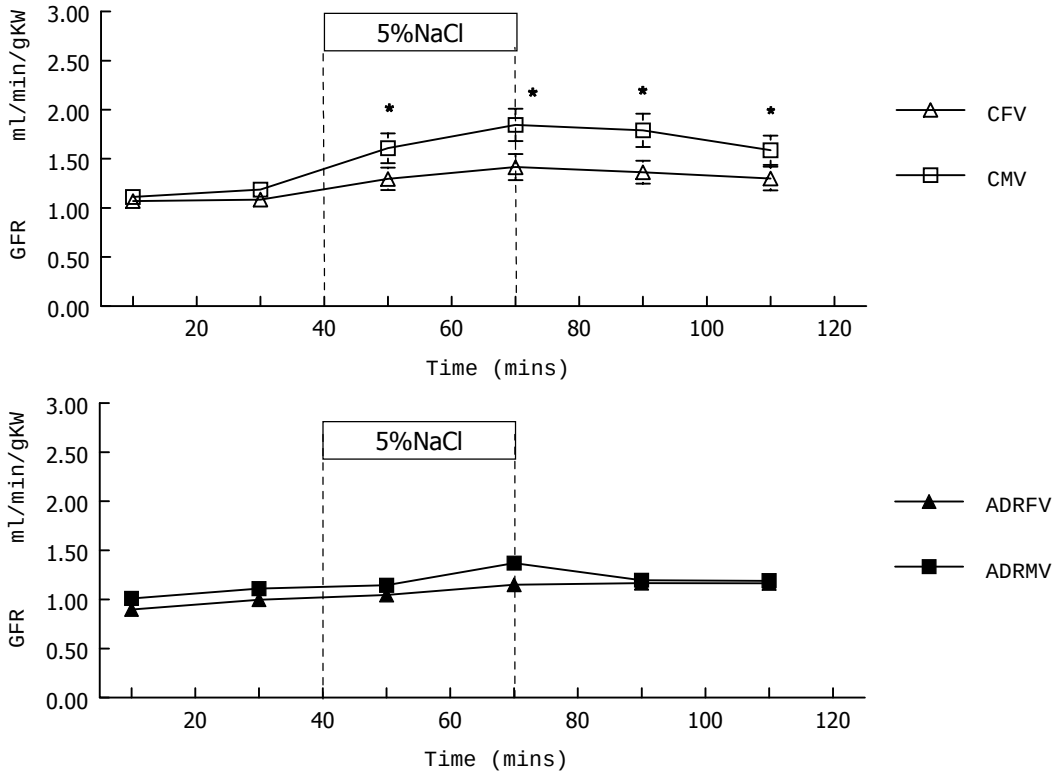

Figure 4. Glomerular filtration rate (GFR) before and after hypertonic saline infusion into either mesenteric vein (MV) or femoral vein (FV) of control and ADR rats. CFV, CMV = control rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; ADRFV, ADRMV = adriamycin treated rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; α, $p < 0.05$ compared with control period in CFV group; *, $p < 0.05$ compared with control period in CMV group; β, $p < 0.05$ compared with control period in ADRFV group; ψ, $p < 0.05$ compared with control period in ADRMV group; N = 8 –10 rats in every group.

Basal efferent renal sympathetic nerve activity increases in nephrotic syndrome (DiBona et al., 1988; DiBona and Kopp, 1997; Herman et al., 1989) and this promotes antinatriuresis (DiBona and Kopp, 1997), potentially leading to sodium retention. Interestingly, acute i.v. isotonic saline

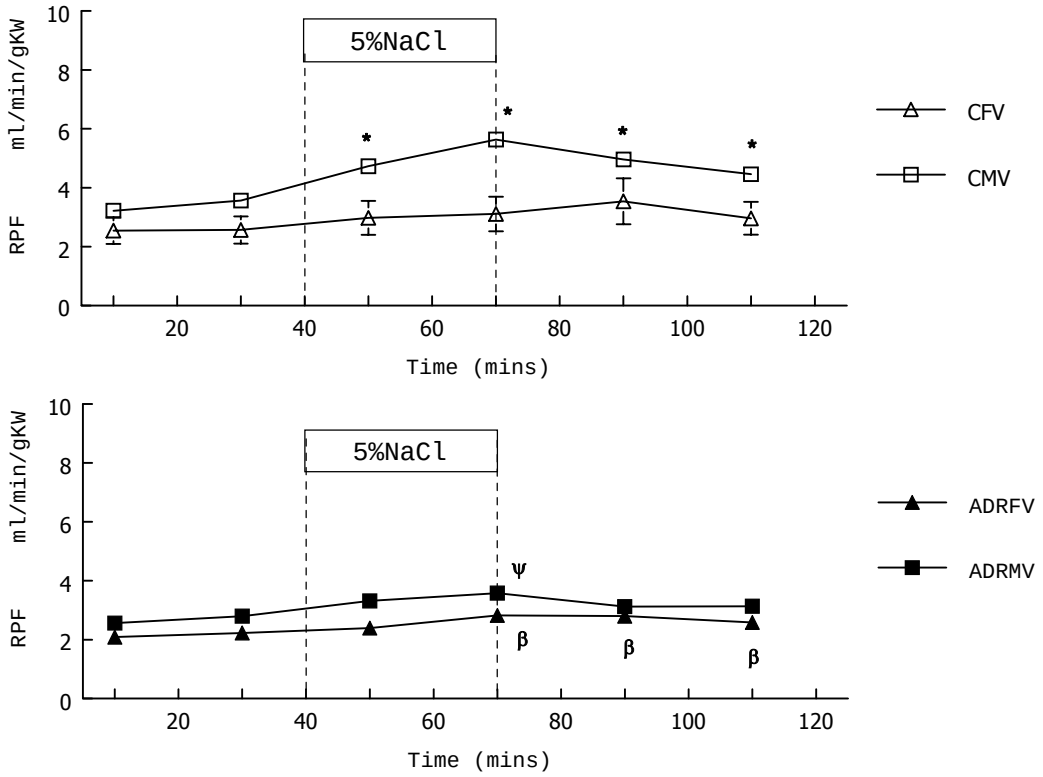

Figure 5. Renal plasma flow (RPF) before and after hypertonic saline infusion into either mesenteric vein (MV) or femoral vein (FV) of control and ADR rats. CFV, CMV = control rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; ADRFV, ADRMV = adriamycin treated rats with hypertonic saline infusion into femoral vein or mesenteric vein, respectively; α, $p < 0.05$ compared with control period in CFV group; *, $p < 0.05$ compared with control period in CMV group; β, $p < 0.05$ compared with control period in ADRFV group; ψ, $p < 0.05$ compared with control period in ADRMV group; N = 8-10 rats in every group.

loading reduces renal nerve activity much less in adriamycin-treated compared to control rats (DiBona et al., 1988). Baroreceptor denervation plus vagotomy and hepatic denervation abolishesthe decrease in renal nerve activity induced by intravenous hypertonic saline load (Morita et al., 1991b). However, the decrease in renal nerve activity induced by oral high NaCl intake is greatly blunted by hepatic denervation alone (Morita et al., 1993). These results suggest that the hepatorenal reflex plays an important role in controlling body fluid homeostasis during food intake. In the current study, infusion of hypertonic saline into the portal vein produced greater sodium excretion than the same infusion into the femoral vein in normal rats, but this response was markedly blunted in the experimental group. A similar finding has also been reported for cirrhotic rat in which the hepatic mechanism for controlling kidney function is impaired in the induction of cirrhosis (Lopez-Novoa and Martinez-Maldonado, 1982). These observations indicate the impairment in the hepatorenal reflex. However, sofar no research has directly tested whether the defect is in the afferent limb, reflex center, or the efferent limb of the hepatorenal reflex. Thus, it should be elucidated by further work. Furthermore, the present data indicate that some other mechanisms, the nervous system and humoral substance are still present in adriamycin-treated rats since small diuresis and natriuresis are still present in these rats. These are important protective mechanisms that tend to reduce the rate of salt and water retention in nephrotic animals.

In conclusion, the present study suggests that a markedly impaired hepatorenal reflex response contributes to salt and water retention in adriamycin-treated rats.

Acknowledgement

The study was supported by a research grant from the Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

References

1. Daly JJ, Roe JW, and Horrocks P. A comparison of sodium excretion following the infusion of saline into systemic and portal veins in the dog: evidence for a hepatic role in the control of sodium excretion. *Clin Sci* 33:481-487, 1967.
2. Davidson WD and Sackner MA. Simplification of the anthron method for the determination of inulin in clearance studies. *J Lab Clin Med* 62:351-356,1963.
3. DiBona GF, Herman PJ, and Sawin LL. Neural control of renal function in edema-forming states. *Am J Physiol* 254:R1017-R1024, 1988.
4. DiBona GF and Kopp UC. Neural control of renal function. *Physiol Rev* 77:75-197, 1997.
5. Haberich FJ. Osmoreceptors in the portal circulation. *Fed Proc 27*(5):1137-1141, 1968.
6. Herman PJ, Sawin LL, and DiBona GF. Role of renal nerves in renal sodium retention of nephrotic syndrome. *Am J Physiol* 256: F823-F829, 1989.
7. Hinojosa-Laborde C, Jones SY, and DiBona GF. Hemodynamics and baroreflex function in rats with nephrotic syndrome. *Am J Physiol* 267:R953-R964, 1994.
8. Ichikawa I, Rennke HG, Hoyer JR, Badr KF, Schor N, Troy JL, Lechene CP, and Brenner BM. Role for intrarenal mechanisms in the impaired salt excretion of experimental nephrotic syndrome. *J Clin Invest* 71:91-103, 1983.
9. Kobashi M and Adachi A. A hepatoportal osmoreceptive afferent projection from nucleus tractus solitarius to caudal ventrolateral medulla. *Brain Res Bull* 24:775-778, 1990.
10. Koepke JP and DiBona GF. Blunted natriuresis to atrial natriuretic peptide in chronic sodium-retaining disorders. *Am J Physiol* 252:F865-F871, 1987.
11. Lopez-Novoa JM and Martinez-Maldonado M. Impaired renal response to splanchnic infusion of hypertonic saline in conscious cirrhotic rats. *Am J Physiol* 242:F390-F394, 1982.
12. Morita H, Ishiki K, and Hosomi H. Effects of hepatic NaCl receptor stimulation on renal nerve activity in conscious rabbits. *Neurosci Lett* 123:1-3, 1991.
13. Morita H, Matsuda T, Furuya F, Khanchowdhury MR, and Hosomi H. Hepatorenal reflex plays an important role in natriuresis after high-NaCl food intake in conscious dogs. *Circ Res* 72:552-559, 1993.
14. Morita H, Nishida Y, and Hosomi H. Neural control of urinary sodium excretion during hypertonic NaCl load in conscious rabbits: role of renal and hepatic nerves and baroreceptors. *J Auton Nerv Syst* 34:157-169, 1991.
15. Morita H, Yamashita Y, Nishida Y, Tokuda M, Hatase O, and Hosomi H. Fos induction in rat brain neurons after stimulation of the hepatoportal Na-sensitive mechanism. *Am J Physiol* 272:R913-R923, 1997.
16. Perico N, Delaini F, Lupini C, Benigni A, Galbusera M, Boccardo P, and Remuzzi G. Blunted excretory response to atrial natriuretic peptide in experimental nephrosis. *Kidney Int* 36:57-64, 1989.
17. Schnaper HW and Robson AM. Nephrotic syndrome: minimal change disease, focal glomerulosclerosis, and related disorders. In: Disease of the kidney and urinary tract, edited by Schrier RW. Philadelphia: Lippincott, Williams & Wilkins, 2001, p.1773-1831.
18. Smith HW. *Principle of renal physiology*. New York: Oxford Press, 1956.

19. Strandhoy JW and Williamson HE. Evidence for an hepatic role in the control of sodium excretion. *Proc Soc Exp Biol Med* 133:419-422, 1970.