Seventh Symposium on Salt, Vol. II 299–304 (1993)
Elsevier Science Publishers B.V., Amsterdam

Role of the Liver in Regulating of Extracellular NaCl Homeostasis

Hironobu Morita, Tsunenori Matsuda, Hideo Ohyama, Masanobu Hagiike, Takao Horiba, Keisuke Miyake, Hideo Yamanouchi and Hiroshi Hosomi

Department of Physiology, Kagawa Medical School, Kagawa, Japan

ABSTRACT

The effects of intravenous or intrahepatic NaCl infusion on renal nerve activity, renal excretory function (hepatorenal reflex) and jejunal absorption (hepatojejunal reflex) were examined.

Role of the renal nerves in the augmentation of Na excretion in response to intravenous NaCl load: We examined responses of renal nerve activity, urine flow and urinary Na excretion to an intravenous hypertonic NaCl infusion in chronically instrumented conscious rabbits with unilateral renal denervation. The intravenous infusion of 20% NaCl, at 0.2 ml/min for 30 min, decreased renal nerve activity by 82 ± 7%. Urine flow and urinary Na excretion increased gradually and peaked at the end of infusion. The innervated kidney excreted significantly greater Na than that in the contralateral denervated kidney.

Hepatorenal reflex: The effects of intraportal infusions of hypertonic solutions on renal nerve activity were examined in chronically instrumented conscious rabbits. A combined infusion of 9% NaCl via the portal vein and distilled water via the inferior vena cava did not alter plasma osmolality or plasma Na concentration of the systemic circulation, but decreased renal nerve activity. The combined infusion of 6.5% LiCl and distilled water did not have any effect on renal nerve activity. The decrease in renal nerve activity induced by the portal 9% NaCl infusion was completely abolished after hepatic denervation. These results indicate that the stimulation of the intrahepatic NaCl receptors, not of the osmoreceptors, elicits a reflex decrease in renal nerve activity.

Hepatojejunal reflex: Jejunal electrolyte absorption was measured in the jejunal loop of anesthetized dogs during the infusions of hypertonic solutions via the portal vein. The net NaCl absorption was not influenced by the vena caval 9% NaCl infusion, although it was significantly attenuated by the portal 9% NaCl infusion. Both hepatic denervation and intravenous atropine injection completely blocked the effects of the portal 9% NaCl infusion on the net NaCl absorption. These results indicate that: (1) net NaCl absorption in dog jejunum is depressed by the hypertonic NaCl infusion via the portal vein; (2) the afferent limb of this response is the hepatic nerves; and (3) the efferent limb of this response is the vagus nerve. Thus, the hepatojejunal reflex may play an important role in the regulation of body fluid homeostasis.

INTRODUCTION

It has been well documented that the mammalian liver is not only a metabolic, clearance, or storage organ but also contains many receptors (Sawchenko, 1979). These involve osmoreceptors, baroreceptors and ionic receptors. Inferring from the role of osmo-, baro-, and ionic receptors in the systemic circulation, these receptors in the hepatic circulation may regulate body fluid homeostasis. In fact, previous works by Daly et al. (1967) and Passo et al. (1972) demonstrated that an infusion of hypertonic NaCl into the portal vein causes a greater natriuresis than similar infusion into the femoral vein, suggesting that the intrahepatic NaCl and/or osmoreceptors control urinary NaCl excretion. However, precise mechanism of this response is still unclear. Furthermore, it is interesting to note that absorbed substances first circulate into the portal and hepatic vasculature before they flow into the systemic circulation and control the kidney output. If this is the case, there is a possibility that the intrahepatic receptors also control the input of NaCl, i.e., intestinal absorption. Accordingly the purpose of this study was: (1) to examine the afferent and efferent pathways of augmentation of urinary Na excretion in response to the intraportal hypertonic NaCl excretion; and (2) to test the hypothesis that intrahepatic NaCl and/or osmoreceptors control jejunal NaCl absorption.

METHODS

Role of renal nerves in augmentation of Na excretion in response to intravenous NaCl load (Morita et al., 1991)

Experiments were conducted in 9 chronically instrumented conscious rabbits weighing 2.0–2.8 kg. The animals were anesthetized with pentobarbital

sodium (30 mg/kg) which was administered intravenously via the ear vein. The right (n = 6) or left (n = 3) kidney was exteriorized through a flank incision and unilateral renal denervation was performed. One week after initial surgery, a second surgery was performed using pentobarbital anesthesia. A venous catheter was inserted into the inferior vena cava for the infusion of hypertonic NaCl solution. An arterial catheter was inserted into the subclavian artery in order to measure arterial pressure. The left (n = 6) or right (n = 3) kidney was exposed retroperitoneally through a flank incision. Renal sympathetic nerves were isolated and two stainless steel electrodes were placed around the nerves. The nerves and electrodes were covered and fixed with silicone gel before closure. The electrodes were exteriorized through the back of the neck. Catheters were implanted into the ureters bilaterally through a suprapubic incision. These catheters were exteriorized and fixed at the inguinal region, then incision was closed. Rabbits were allowed to recover from surgical stress for at least 2 days before the experiments were begun.

All experiments were conducted in conscious rabbits that were placed in a box which loosely restricted their movement. Arterial pressure, heart rate and renal nerve activity were monitored continuously. After a stabilization period, a 30-min control period was started. At the end of the control period, the infusion of 20% NaCl, at a rate of 0.2 ml/min, was carried out for 30 min. This was followed by a 30-min recovery period. Urine was collected into test tubes by gravity drainage at 10-min clearance intervals through the bilaterally inserted ureter catheters. Thus, urine samples from the innervated and contralateral denervated kidney were collected separately.

Hepatorenal reflex (Morita et al. 1991)

Experiments were conducted in 16 chronically instrumented conscious rabbits weighing 2.2–2.8 kg. The animals were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). Through the central laparotomy, a portal catheter for the infusion was inserted via the mesenteric vein. In 8 of 16 rabbits, the hepatic nerves were sectioned. There are two main neural pathways that supply the liver: a plexus around the hepatic artery (anterior plexus) and one on the portal vein (posterior plexus) (Pick, 1970). To achieve hepatic denervation, these two plexuses were sectioned and connective tissue around the hepatic artery, portal vein and bile duct was also sectioned. A venous catheter was inserted into the inferior vena cava via the right jugular vein and an arterial catheter was inserted into the aorta via the left subclavian artery. The catheters were exteriorized through the back of the neck; then the incisions were closed. After the surgery the rabbits were given penicillin intramuscularly for 3 days. One week after initial surgery, a second surgery was performed using pentobarbital sodium (30 mg/kg) anesthesia. Through a flank incision, the renal nerves along the renal artery were isolated and two stainless steel electrodes were placed around the nerves. The nerves and electrodes were covered and fixed with silicone gel before closure. The electrodes were exteriorized through the back of the neck. Rabbits were allowed to recover from surgical stress for at least 2 days before the experiments were begun.

All experiments were conducted in conscious rabbits that were placed in a box which loosely restricted their movement. Arterial pressure, heart rate and renal nerve activity were monitored continuously. A hypertonic solution of 9% NaCl or 6.5% LiCl (0.1 ml/min) was infused for 10 min via the portal vein while distilled water (0.9 ml/min) was infused via the inferior vena cava. Then the infusion route was switched: NaCl or LiCl was infused via the inferior vena cava while distilled water was infused via the portal vein. A 30–60 min stabilization period was observed before each infusion. In this "double-infusion" technique, the total solution introduced into the systemic circulation would be isotonic. The order of the solution and infusion route was randomized. Arterial blood samples (1.0 ml) for measuring plasma Na concentration and plasma osmolality were taken just before the beginning and the end of the infusion.

Hepatojejunal reflex (Morita et al., 1990)

All experiments were conducted in 31 mongrel adult dogs weighing 9–16 kg. Dogs were deprived of food 24 h before experiments. Water remained available throughout the food-deprivation period. Dogs were anesthetized with pentobarbital sodium (30 mg/kg). A venous catheter was inserted into the inferior vena cava via the femoral vein. An arterial catheter was inserted into the abdominal aorta via the femoral artery. Through the central laparotomy, the jejunal loop was made, which was 30 cm long and started from 10 cm distal to the ligament of Treitz. The loop was washed with warm saline and intubated in both ends of loop. A portal catheter was inserted via the mesenteric vein. The intestine was returned to the abdominal cavity. The tubes were exteriorized and the incision was closed.

A 30–60 min equilibration period was observed before the experiment. To examine the jejunal electrolyte absorption, the test solution (30 ml, 37°C) was injected into the jejunal loop from the proximal tube

and allowed to remain for 15 min, then it was collected from the distal tube. The test solution had the following composition (in mEq/l): Na 130; K 4; Ca 3; Cl 109; CH_3COO 28; and glucose 50 g/l. The volume and Na, K, Cl concentration of injected and collected fluid were measured. Water and electrolyte net absorption were calculated as the difference between the absolute values of the injected solution and the collected solution.

Animals were divided into four groups. In group 1 (n = 7), jejunal net electrolyte absorption was measured with and without the infusion on 9% NaCl or 6.5% LiCl via the portal vein or the inferior vena cava. In group 2 (n = 8), net absorption was measured during the infusion of 9% NaCl or 50% glucose via the portal vein or the inferior vena cava. In group 3 (n = 8), net absorption was measured before and after hepatic denervation with or without the portal 9% NaCl infusion. In group 4 (n = 8), net absorption was measured before and after the bolus injection of atropine (0.5 mg/kg) with or without the portal 9% NaCl infusion. In all groups, the infusion of the hypertonic solution (0.02 ml/kg/min) was initiated 10 min before the injection of the test solution into the jejunal loop and lasted for 25 min. A 20 min equilibration period was allowed between the infusions.

All values presented here are reported as means ± SE. For statistical analysis a two-way analysis of variance was used. When the F ratio exceeded the critical value, Dunnett's multiple-sample test was applied to test the significance of the difference. $P<0.05$ was taken as the criterion for the significant difference.

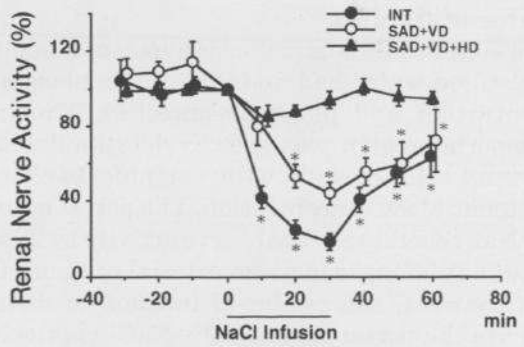

Fig. 1. Responses of renal nerve activity to hypertonic NaCl infusion are illustrated. Filled circles represent responses in intact rabbits, open circles represent responses in rabbits with SAD plus vagotomy, and triangles represent responses in rabbits with SAD plus vagotomy plus hepatic denervation. *P<0.05, significantly different from pre-infusion control values.

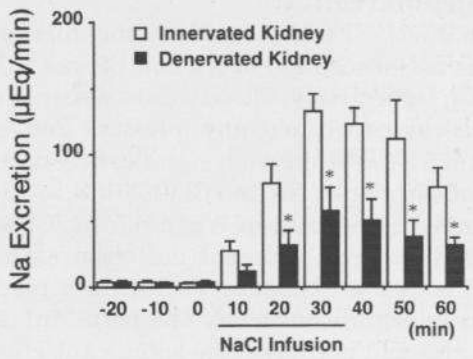

Fig. 2. Comparison of responses of urinary Na excretion to hypertonic NaCl infusion in conscious rabbits with unilateral renal denervation. *P<0.05, significantly different from the innervated kidney.

RESULTS

Role of renal nerves in augmentation of Na excretion in response to intravenous NaCl load

Intravenous infusion of hypertonic NaCl increased plasma Na concentration by an average of 16 ± 3 mEq/l, from 138 ± 2 to 154 ± 2 mEq/l and plasma osmolality by an average of 27 ± 5 mOsm/kg, from 289 ± 3 to 316 ± 4 mOsm/kg. These changes were accompanied by a marked decrease in renal nerve activity, i.e., renal nerve activity decreased instantaneously with the hypertonic NaCl infusion and reached a minimum value of 19 ± 4% at the end of the infusion period (Fig. 1). Renal nerve activity gradually recovered toward the control level, but at the end of the recovery period, a small yet significant reduction of 37 ± 9% was still observed.

Responses of urinary Na excretion to NaCl infusion are illustrated in Fig. 2. The control values of the urine flow (0.08 ± 0.03 ml/min) and urinary Na excretion (3.8 ± 0.8 μEq/min) from the denervated kidney were not significantly different from those of the contralateral innervated kidney (0.05 ± 0.01 ml/min and 3.7 ± 0.7 μEq/min, respectively). Diuresis and natriuresis occurred almost instantaneously with the hypertonic NaCl infusion and reached a peak at the end of the infusion period. However, the responses in the denervated kidney were quantitatively different from the responses in the contralateral innervated kidney. At the end of the infusion period, urine flow from the innervated kidney increased to 0.59 ± 0.07 ml/min, which was significantly more than that from the denervated kidney (0.23 ± 0.06 ml/min). In the innervated kidney, Na excretion increased to 132.6 + 13.3 μEq/min, while in the denervated kidney Na excretion increased only to 57.4 ± 17.9 μEq/min ($p<0.05$).

Hepatorenal reflex

The simultaneous infusion of hypertonic solution and distilled water had no influence on plasma Na concentration and plasma osmolality. Thus, the hepatoportal region was selectively stimulated by hypertonic solution without any significant changes in systemic blood concentration. The portal infusion of 9% NaCl decreased renal nerve activity by 29 ± 3% without any changes in mean arterial pressure (Fig. 3). In contrast, the combined infusion of distilled water via the portal vein and 9% NaCl via the inferior vena cava had no influence on renal nerve activity. The combined infusion of 6.5% LiCl and distilled water also had no effect on renal nerve activity. In hepatic denervated rabbits, the decrease in renal nerve activity during the combined infusion of 9% NaCl via the portal vein and distilled water via the inferior vena cava was completely abolished.

Hepatojejunal reflex:

When 9% NaCl was infused via the inferior vena cava, the net absorption of Na and Cl was 1.34 and 1.05 mEq, respectively. Those values were not different from values without any infusion. Conversely, the portal 9% NaCl infusion significantly decreased the net absorption of Na and Cl (0.29 ± 0.16 and 0.13 ± 0.16 mEq, respectively). When 6.5% LiCl was infused via the portal vein, the net absorption of Na and Cl tended to decrease but did not reach the statistically significant level. The portal infusion of 50% glucose did not have any significant effects on the net absorption of Na and Cl. To examine the afferent pathway of the decreased net absorption during the portal infusion of 9% NaCl, the net absorption of Na and Cl were examined before and after hepatic denervation. The decrease in net absorption of Na and Cl induced by the portal 9% NaCl infusion was completely abolished by hepatic denervation (Figure 4). To examine the efferent pathway of the decreased net absorption during the portal infusion of 9% NaCl, the net absorption was examined before and after an intravenous injection of atropine. Atropine completely abolished the decrease in net absorption induced by the portal 9% NaCl infusion (Fig. 4).

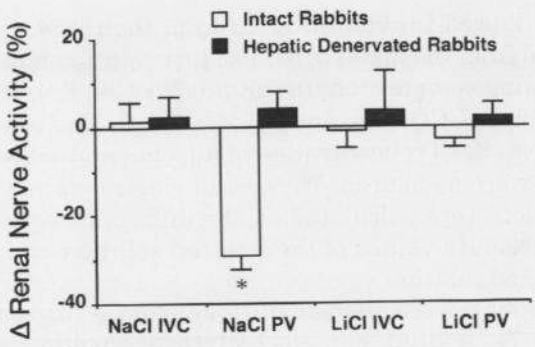

Fig. 3. Changes in renal nerve activity to portal venous (PV) or inferior vena caval (IVC) infusion of 9% NaCl or 6.5% LiCl are illustrated. *P<0.05, significantly different from control.

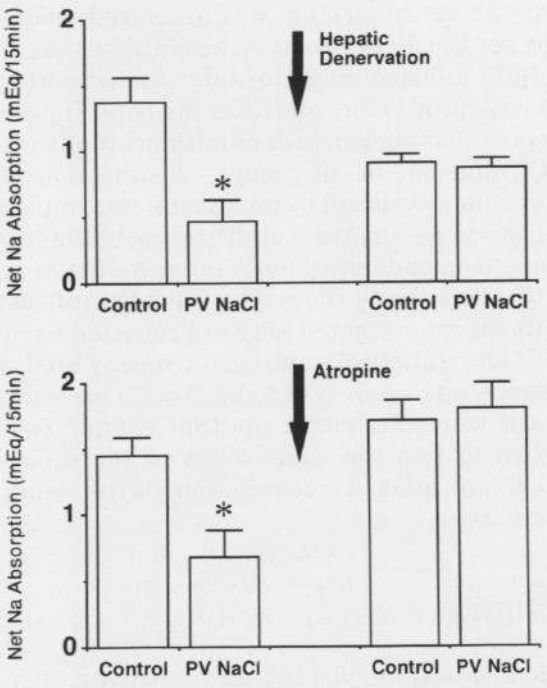

Fig. 4. Effects of 9% NaCl infusion via the portal vein on net Na absorption before and after hepatic denervation (upper panel) and atropine injection (lower panel). *P<0.05, significantly different from control.

DISCUSSION

The major findings of the present study are: (1) the intravenous hypertonic NaCl infusion elicits the decrease in renal nerve activity, which has a significant role in augmentation of urinary Na excretion in response to NaCl load; (2) the hypertonic NaCl infusion into the portal vein elicits reflex decreases in renal nerve activity and jejunal net absorption of Na and Cl; (3) these responses may not be due to an osmotic stimulus to the hepatoportal region; (4) the afferent pathway of these reflexes is the hepatic nerves; and (5) the efferent pathways of these reflexes include the renal sympathetic nerves and the cholinergic fibers to the jejunum.

Intravenous infusion of hypertonic NaCl solution elicited not only the increases in plasma NaCl concentration and osmolality but also the decrease in hematocrit. This suggests that the hypertonic stimulus elicited a shift of interstitial fluid into the vessels. If this is the case, then the infusion of hyper-

tonic NaCl solution elicits not only osmotic, Na, and Cl load but also a volume load. A volume load with isotonic saline decreased renal nerve activity, which was mediated predominantly by the vagus nerve (Morita et al., 1985). However, the decrease in renal nerve activity in the present study was not only due to the volume load, since the decrease in renal nerve activity induced by the NaCl load was not abolished by sinoaortic baroreceptor denervation plus vagotomy (Morita et al., 1991). Combined hepatic denervation and sinoaortic baroreceptor denervation plus vagotomy completely abolished the decrease in renal nerve activity (Morita et al., 1991). Thus, the hepatic nerves mediate the decrease in renal nerve activity in response to the hypertonic NaCl infusion. Furthermore, Vallet and Baertschi (1980) demonstrated that the superinfusions of the portal vein with hypertonic NaCl increases neural activity in the hypothalamo-neurohypophysial tract. These results indicate that the hepatic NaCl receptor affect renal NaCl and water excretion by altering renal nerve activity and neurohypophysial hormone release.

The simultaneous infusion of hypertonic solution and distilled water did not alter plasma Na concentration and osmolality of the systemic circulation but elicited a small decrease in hematocrit (–0.9 ± 0.3%). Thus, the simultaneous infusion elicited not only a selective stimulation on the hepatic receptors but also a volume load. Volume load itself decreases renal nerve activity (Morita et al., 1985). However, the decrease in renal nerve activity in the present study could not have been due to a volume load, since renal nerve activity did not decrease when distilled water was infused intraportally and 9% NaCl was simultaneously infused intracavally. In both cases, the same volume (10 ml/10 min) was infused. Moreover, the decrease in renal nerve activity could not have been due to the osmotic stimulus on the hepatoportal region, since equiosmotic LiCl did not have a significant effect on renal nerve activity. Thus, we believe that the intrahepatic receptors which reflexly decrease renal nerve activity may be NaCl sensitive.

In the present study, we demonstrated that the hepatic osmo- and/or ionic receptor reflexly controls jejunal Na and Cl absorption and/or secretion. This reflex occurred when the intrahepatic receptors were stimulated by hypertonic NaCl but not by equiosmotic glucose. This suggests that intrahepatic receptors of this reflex may not be osmosensitive but rather sensitive to changes in Na or Cl ion concentration. The intraportal equiosmotic LiCl administration tended to decrease jejunal net absorption of Na and Cl, although it did not reach significant level. Moreover, net Na and Cl absorption during the portal LiCl infusion were not significantly different from these during the portal NaCl infusion. Thus, we cannot rule out the possibility that osmolality may be the stimulus.

The existence of ion-sensitive receptors in the liver is supported by electrophysiological observations of Andrews and Orbach (1974). They have recorded afferent nerve activity in the rabbit liver, which responded to alterations of NaCl concentration but not to those of sucrose, glucose, or mannitol. However, in their study, LiCl was similarly effective.

To examine the efferent pathway of the decreased net absorption, jejunal net absorption was measured before and after the intravenous injection of atropine. Atropine completely blocked the decrease in net NaCl absorption induced by the portal 9% NaCl infusion, suggesting that acetylcholine is the nerve transmitter of the efferent pathway. Although we did not measure parasympathetic nerve activity to the jejunum, it may have increased during the portal infusion of 9% NaCl. In support of this hypothesis, electrical and chemical stimulation of the dorsal motor nucleus of the vagus nerve decreased water absorption and the response was abolished by cervical vagotomy (Martin et al., 1989).

CONCLUSIONS

In conclusion, we have provided the evidence that an intrahepatic ionic receptor affects renal NaCl excretion and jejunal NaCl absorption. The afferent pathway is the hepatic nerve and the efferent pathways includes the renal sympathetic nerve and cholinergic fibers to the jejunum. Orally ingested and intestinally absorbed NaCl first circulate through the portal and hepatic vasculature, then extracellular NaCl homeostasis may be controlled by altering intestinal absorptive and renal excretory functions before systemic NaCl concentration is altered. These observations introduce the interesting concept that the body fluid regulating system starts its operation before the composition of systemic blood is altered.

ACKNOWLEDGEMENTS

Dr. Morita was supported by Grant-in Aid for Encouragement of Young Scientist of Japan (No. 03770043). This study was supported in part by the Research Grant for Cardiovascular Disease 3A-1 from the Ministry of Health and Welfare of Japan and a research grant from The Salt Science Research Foundation.

REFERENCES

Andrews, W.H.H. and Orbach, J., 1974. Sodium receptors activating some nerves of perfused rabbit livers. Am. J. Physiol., 227: 1273–1275.

Daly, J.J., Roe, J.W. and Horrocks, P., 1967. 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.

Martin, K., Kong, T.H., Renehan, W., Schurr, A., Dong, W.. Zhang, X. and Fogel, R., 1989. Identification and function of brain stem neurons regulating rat ileal water absorption. Am. J. Physiol., 257: G266–G273.

Morita, H. and Vatner, S.F., 1985. Effects of volume expansion on renal nerve activity, renal blood flow and sodium and water excretion in conscious dogs. Am. J. Physiol., 249: F680–F687.

Morita, H., Ohyama, H., Hagiike, M., Horiba, T., Miyake, K., Yamanouchi, H., Matsushita, K. and Hosomi, H., 1990. Effects of portal infusion of hypertonic solution on jejunal electrolyte transport in anesthetized dogs. Am J. Physiol., 259: R1289–R1294.

Morita, H., Ishiki, K. and Hosomi, H., 1991. Effects of hepatic NaCl receptor stimulation on renal nerve activity in conscious rabbits. Neurosci. Lett., 123: 1–3.

Morita, H., Nishida, Y. and Hosomi, H., 1991. 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: 157–170.

Passo, S.S., Thornborough, J.R. and Rothballer, A.B., 1972. Hepatic receptors in control of sodium excretion in anesthetized cats, Am. J. Physiol., 224: 373–375.

Pick, J., 1970. Autonomic Nervous System. Lippincott, Philadelphia, PA pp. 324–327.

Sawchenko, P.E. and Friedman, M.I., 1979. Sensory function of the liver — a review. Am. J. Physiol., 236: R5–R20.

Vallet, P. and Baertschi, A.J., 1980. Sodium-chloride sensitive receptors located in hepatic portal vein of the rat. Neurosci. Lett., 17: 283–288.