**Effects of High NaCl Diet on Arterial Pressure in Sprague-Dawley Rats with Hepatic and Sinoaortic Denervation**

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**Abstract:** The Na\(^+\) receptor that exists in the hepatoportal region plays an important role in postprandial natriuresis and the regulation of Na\(^+\) balance during NaCl load. Thus it would be considered that a dysfunction of the hepatic Na\(^+\) receptor might result in the elevation of arterial pressure under a condition of high NaCl diet. To elucidate this hypothesis, arterial pressure was continuously measured during three weeks of high NaCl diet (8\% NaCl) in four groups of rats: (i) intact rats, (ii) rats with hepatic denervation (HD), (iii) rats with sinoaortic denervation (SAD), and (iv) rats with SAD+HD. During a 1-week normal NaCl diet period, there was no difference in arterial pressure among the four groups. A high NaCl diet had no influence on arterial pressure in intact or HD rats; however, it significantly increased by 11 ± 3 mmHg in SAD rats. The addition of HD to SAD had no synergistic effect on arterial pressure; i.e., in SAD+HD rats, mean arterial pressure increased by 13 ± 1 mmHg. In conclusion, sinoaortic baroreceptor, but not hepatic Na\(^+\) receptor, has a significant role in the long-term regulation of arterial pressure on a high NaCl diet. [The Japanese Journal of Physiology 55: 229–234, 2005]

**Key words:** hepatic denervation, sinoaortic denervation, high NaCl diet, arterial pressure, telemetry system.

Previous studies by our laboratory have demonstrated that the hepatic Na\(^+\) receptor plays an important role in controlling Na\(^+\) excretion from the kidney [5–7, 9]. Rats receiving a continuous intravenous infusion of hypertonic NaCl, i.e., bypassing the hepatic receptors, retain more Na\(^+\) than rats with intraportal infusion; this difference is seen only during the first 1 or 2 days, but not thereafter [9]. The difference is seen both in Dahl salt-sensitive rats and in Dahl salt-resistant rats. However, the increased Na\(^+\) balance results in the elevation of arterial pressure only in Dahl salt-sensitive rats, not in Dahl salt-resistant rats. Furthermore, Carlson *et al.* [1] demonstrated that arterial pressure of the hepatic denervated Wistar-Kyoto rats is significantly higher than that of the sham-operated rats on a normal NaCl diet; however high NaCl diet does not increase arterial pressure above baseline levels in either hepatic denervated rats or sham-operated rats. Thus under the NaCl load, the hepatic Na\(^+\) receptor might have a significant role in long-term arterial pressure regulation in the salt-sensitive strain, but not in the salt-resistant strain. However, it should be noted that arterial pressure is controlled by many factors, the most important one being the sinoaortic baroreceptor [3]. Therefore observed arterial pressure might be the result of having already been buffered by baroreflex. There is a possibility that arterial pressure in hepatic denervated salt-resistant rats may be increased by a high NaCl diet unless the sinoaortic baroreflex operates. So the role of the hepatic Na\(^+\) receptor in controlling arterial pressure should be estimated in rats without a sinoaortic baroreceptor. For this purpose,
arterial pressure was continuously measured by a chronically instrumented radiotelemetry transducer in freely moving salt-resistant Sprague-Dawley rats with sinoaortic baroreceptor denervation and hepatic denervation. Methods

This study was approved by the Animal Care and Use Committee of our institution. The rats were maintained in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan. Male Sprague-Dawley rats were purchased from Charles River Japan at 8 weeks of age. All rats were maintained at constant humidity (60 ± 5%), temperature (23 ± 1°C), and light cycle (07:00–19:00 h). One week after arrival, the rats were housed in individual metabolic cages, and another week elapsed prior to surgery. The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and randomly divided into four groups: intact (n = 7), hepatic denervation (HD, n = 6), sinoaortic baroreceptor denervation (SAD, n = 6), and SAD+HD (n = 7). For SAD, with the aid of a surgical microscope the aortic depressor nerves were bilaterally isolated and severed by a midcervical incision. The region of the carotid bifurcation was then stripped and painted with 10% phenol in ethanol (n = 13). Sham SAD was performed on the 13 rats in the intact and HD groups. After surgery, an antibiotic was administered for 3 days to prevent infection.

One week after the SAD or sham surgery, an implantation of a telemetry transmitter was performed. Under pentobarbital anesthesia (50 mg/kg, I.P.), the abdominal aorta was exposed via a midline laparotomy. The catheter part of the telemetry transmitter probe for AP measurement (TAP-C40, Data Sciences International, St. Paul, MN) was inserted into the abdominal aorta. The tip of the catheter was set distal to the renal artery bifurcations, and the probe was then sutured to the abdominal wall. In 6 of 13 sham rats and 7 of 13 SAD rats, HD was performed by severing the periaortal hepatic nervous plexus and stripping the portal vein and bile duct; 10% phenol in ethanol was then applied around the hepatic artery, portal vein, and bile duct. Sham surgery was performed by using the same procedure as HD, whereas the periaortal hepatic nervous plexus was left intact and applied with saline instead of phenol. After surgery, an antibiotic was administered for 3 days. The rats were returned to their home cage, and another week elapsed for recovery from the surgery. A 0.4% NaCl diet and distilled water were provided ad libitum throughout the one-week recovery period.

After that, a 28-day experimental period was started. The outputs of the drink and pellet counters were continuously recorded on a computer via an interface (PAW-2500, MATYS, Toyo Sangyo, Toyama, Japan), thus allowing a continuous monitoring of water and pellet consumptions. The transmitter signal was continuously monitored by a receiver over the cage that was connected to a computer. Data acquisition and analysis were performed with Dataquest IV software (Data Sciences International, St. Paul, MN). The arterial pressure was sampled for 10 s every 5 minutes, and the heart rate was determined from the pressure waveform. Mean arterial pressure and heart rate data were then calculated and stored for later analyses. Body weight, urine volume, and urinary Na⁺, K⁺, and Cl⁻ excretion were measured daily throughout the ex-
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The experiment, which was divided into two periods: normal NaCl diet (days 1–7, 0.4% NaCl diet) and high NaCl diet (days 8–28, 8% NaCl diet).

All values presented are means ± SE. The data in Fig. 4 were analyzed by two-way ANOVA, with the groups of rats (intact, HD, SAD, and SAD+HD) and diets (normal NaCl and high NaCl) as factors. If the F ratio indicated a statistical significance, a post hoc test was applied to compare the among-group and within-group means. The significant level of the post hoc comparisons was set at $P < 0.05$.

Results

Figures 1–3 show the daily responses of body weight, food intake, water intake, urinary volume, urinary Na+, K+, and Cl− excretion, mean arterial pressure, and heart rate. For statistical analyses, the averaged data of days 1–7 (normal NaCl diet) and days 22–28 (high NaCl diet) were presented in Fig. 4.

On the first day of the experiment, the body weight of the intact group (379 ± 15 g) tended to be larger compared to the HD (356 ± 10 g), SAD (345 ± 8 g), and SAD+HD (347 ± 8 g) groups; however, the difference did not reach statistical significance. During the 4-week experimental period, body weight increased

Fig. 2. Responses of urine volume, urinary Na+, K+, and Cl− excretion in intact ($n = 7$), hepatic denervated (HD, $n = 6$), sinoaortic baroreceptor denervated (SAD, $n = 6$), and SAD+HD ($n = 7$) rats.

Fig. 3. Responses of mean arterial pressure and heart rate in intact ($n = 7$), hepatic denervated (HD, $n = 6$), sinoaortic baroreceptor denervated (SAD, $n = 6$), and SAD+HD ($n = 7$) rats.
Fig. 4. Averaged data of 1–7 days (normal NaCl diet period) and 22–28 days (the last week of a high NaCl diet) of food intake, water intake, urine volume, urinary Na⁺, K⁺, and Cl⁻ excretion, mean arterial pressure, and heart rate. *P < 0.05 from normal diet period. †P < 0.05 from intact rats.
prosperously in all groups (Fig. 1). Food intake was significantly suppressed by high NaCl diet in the intact and HD groups, and in the SAD and SAD+HD groups, the difference did not reach statistical significance (Fig. 4). During the high NaCl diet period, food intake in the SAD+HD group tended to be higher compared to the intact group; however, the difference did not reach statistical significance. Water intake, urine volume, and Na⁻ and Cl⁻ excretions were increased by the high NaCl diet, whereas K⁺ excretion was depressed.

Mean arterial pressure under normal diet in the SAD and SAD+HD groups seems to be higher than in the intact and HD groups; however, the difference did not reach statistical significance. In the intact and HD groups, the high NaCl diet did not alter mean arterial pressure, whereas in the SAD and SAD+HD groups the mean arterial pressure gradually increased (Fig. 3). During the last week of the high NaCl diet period, mean arterial pressure increased to 110 ± 3 mmHg, from 101 ± 2, in the SAD+HD group (Fig. 4). During this period, mean arterial pressures in the SAD and SAD+HD groups were significantly higher than in the intact (93 ± 1 mmHg) and HD (92 ± 1 mmHg) groups. In the SAD and SAD+HD groups, the heart rate was not altered by the high NaCl diet, whereas it gradually decreased in the intact and HD groups during the high NaCl diet period (Fig. 3). During the last week of the high NaCl diet period, heart rates in the intact and HD groups decreased to 330 ± 8 and 317 ± 3 bpm, respectively, which were significantly lower compared to the SAD (368 ± 13 bpm) and SAD+HD (366 ± 7 bpm) groups.

Discussion

The major findings of the present study: (i) SAD itself did not alter arterial pressure; however, the combination of SAD and a high NaCl diet significantly increased arterial pressure; (ii) the addition of HD to SAD had no synergistic effect on arterial pressure. It is well documented that the sinoaortic baroreceptor plays an important role in the moment-to-moment stabilization of arterial pressure, but not in the long-term level of arterial pressure [2, 11, 13]. Cowley et al. [2] demonstrated the tremendous variation in arterial pressure in SAD dogs, but SAD does not cause a significant change in the long-term level of arterial pressure. Thus the sinoaortic baroreceptor has been considered not to have an important role in long-term arterial pressure regulation. However, under conditions of high dietary NaCl intake, the role of sinoaortic baroreceptor in long-term arterial pressure regulation becomes critical [4, 12]. The results of the present study are quite consistent with the previous observation. A high NaCl diet significantly increased mean arterial pressure in the SAD rats (+11 ± 3 mmHg), but not in the intact rats. Furthermore, in intact rats the heart rate was significantly decreased by the high NaCl diet (−28 ± 4 bpm); however a decrease in heart rate was not observed in SAD rats, and the decrease in heart rate might be mediated via baroreflex. These results suggest that under the conditions of a high NaCl diet, baroreflex operates to prevent an elevation of arterial pressure.

We have demonstrated that the hepatic Na⁺ receptor plays an important role in postprandial natriuresis; this is based on experimental observations in which a disruption of the hepatic receptors reduced the natriuresis on the oral NaCl load [5, 6, 14]. Thus it is possible that a disruption of the hepatic Na⁺ receptor and the NaCl load would result in an elevation of arterial pressure as a result of a positive Na⁺ and water balance. This is true with Dahl salt-sensitive rats. That is, the role of hepatic Na⁺ receptors on controlling the Na⁺ balance is more significant in salt-sensitive rats than in salt-resistant rats, and the combination of a disruption of the hepatic Na⁺ receptor and the NaCl load results in an elevation in arterial pressure in salt-sensitive rats, but not in salt-resistant rats [9]. Furthermore, Carlson et al. [1] chronically and continuously measured arterial pressure with a telemetry system and found that mean arterial pressure in HD Wistar-Kyoto rats is higher than in sham-operated rats on a normal NaCl diet, but it does not increase further on a high NaCl diet. In the present study, however, the HD itself did not increase mean arterial pressure. This difference is probably due to the strain difference between Wistar-Kyoto and Sprague-Dawley and/or the difference of HD procedures. In the HD procedures, it is important that only the Na⁺-sensitive function is destroyed and that the other hepatic function is preserved. However, there is no solid evidence that the all-hepatic Na⁺-sensitive function was completely destroyed by the HD procedure we employed in the present study. This is the limitation of the present study. However, a previous study at our laboratory demonstrated that the periarterial hepatic nerve, which we severed, is the major afferent that mediates an increase in portal venous Na⁺ concentration [10]. Furthermore, the HD procedure abolishes Fos expression induced by portal venous hypertonic NaCl infusion [8]. Thus we believe that most of the central projection from the hepatic Na⁺ receptor was blocked by the HD procedure.
In the present study, the role of hepatic Na\(^+\) receptor on mean arterial pressure was also examined in the SAD rats, since baroreflex might buffer the altered arterial pressure. However, the addition of HD to SAD had no synergistic effect. The possible explanation for no effect of HD on arterial pressure is that a long-term oral load of high NaCl diet decreases an expression of Na\(^+\) receptor in the liver and then causes a reduction in hepatic Na\(^+\) receptor sensitivity [15]. Thus the role of the hepatic Na\(^+\) receptor in controlling renal Na\(^+\) excretion under the condition of high oral NaCl load might be a short-term effect, but not a long-term one. In fact, rats receiving a continuous intravenous infusion of hyperosmic NaCl, i.e., bypassing the hepatic receptors, retain more Na\(^+\) than rats with intraportal infusion, but this difference is seen only during the first 1 or 2 days [9].

In summary, continuous and chronic measurement of arterial pressure using a telemetry system reveals that a combination of SAD and dietary NaCl load increases mean arterial pressure. However, the addition of HD has no appreciable synergistic effect on arterial pressure.

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REFERENCES