Role of the liver in long-term control of drinking behavior, Na\(^+\) balance, and arterial pressure in Dahl rats

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Morita, Hironobu, Kiyoshi Tsunooka, Masanobu Hagiike, Osamu Yamaguchi, and Ken Lee. Role of the liver in long-term control of drinking behavior, Na\(^+\) balance, and arterial pressure in Dahl rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1111–R1118, 1998.—The role of postabsorptive mechanisms in long-term control of drinking behavior, Na\(^+\) balance, and arterial pressure was examined in Dahl salt-sensitive (DS) and salt-resistant (DR) rats. NaCl (0.15 M) was infused (0.5 ml/h) into either the inferior vena cava (IVC) or the portal vein (PV) for 7 days, and then 1.5 M NaCl was infused for 10 days. During 1.5 M infusion, the IVC group retained more Na\(^+\) than the PV group. Furthermore, in DS rats, mean arterial pressure was higher in the IVC group than in the PV group. Regardless of the strain and infusion route, 1.5 M infusion had no effect on volume of daily saline consumption. However, when the data for light and dark periods were analyzed separately, dark period saline consumption in the PV group was decreased by 1.5 M infusion but was not changed in the IVC group. These results indicate that, in Dahl rats, the postabsorptive mechanism plays a significant role in controlling long-term saline drinking behavior and Na\(^+\) balance and has a significant role in controlling arterial pressure in DS, but not DR, rats.

METHODS

Thirty-two male Dahl rats [DS: n = 16; salt resistant (DR): n = 16, body wt 230–285 g, 9 wk old, Seiwa LabAnimal] were used; they were kept under conditions in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan.

The experimental protocol is presented schematically in Fig. 1. Five days before the operation, the rats were housed in individual metabolic cages with a 12:12-h light-dark cycle and were fed low-NaCl pellet (Na\(^+\) 0.0075 mmol/g food), distilled water and isotonic saline being provided ad libitum, using a "two-bottle" self-selection procedure, the bottles being equipped with drop counters, which continually measured water and saline consumption; these conditions were maintained throughout the experiment. After measurement of body weight and systolic arterial pressure (tail cuff method), the first operation was performed. Under ether anesthesia and sterile conditions, the abdomen was entered by midline laparotomy. A Silastic catheter connected to polyethylene tubing (PE-50) was placed, via the splanchnic vein, in the PV, pointing toward the liver, while an IVC catheter was inserted via the femoral vein. The catheters were exteriorized through the midscapular region, and the incisions were closed. A plastic connector was implanted in the midscapular region for protecting the catheters and for connecting to a flexible spring. The rats were returned to their cages and were given postoperative care and monitored to ensure that food and water intake had returned to presurgical levels.

Three days after the operation, one of the catheters (PV or IVC) was extended and passed along the interior of a flexible spring connected to the plastic connector protecting the catheters. The spring and catheter were then connected to a swivel arrangement placed above the metabolic cage, allowing unrestricted movement. PV or IVC infusion of sterile 0.15 M NaCl solution (12 ml/day by syringe pump; KDS 220,
KD Scientific) was started and continued for 7 days; then, infusing solution was changed to 1.5 M NaCl for a further 10 days. Measurement of daily water and saline intake and urine output started on day 3 of 0.15 M NaCl infusion and continued until day 7 of 1.5 M NaCl infusion. The output of the drink counters was continuously recorded on a computer via an interface (PAW-2500, MATYS; Toyo Sangyo, Toyama, Japan), thus allowing both overall and continuous monitoring of water and saline consumption by direct volume measurement (used to analyze the daily intake) and drop counting (used to analyze light- and dark-period drinking behavior), respectively. The Na⁺ concentration of the infusion solution, drinking solution, and urine was measured by flame photometry (Hitachi 750, Tokyo, Japan). Because the food contains very low levels of Na⁺ (0.0075 mmol/g), fluid and Na⁺ balances were calculated from the difference between daily fluid intake (PV or IVC infusion plus drink) and urinary output.

On day 8 of 1.5 M NaCl infusion, an arterial catheter was inserted into the abdominal aorta via the femoral artery under ether anesthesia. On day 10 of 1.5 M NaCl infusion, the arterial pressure was measured in the conscious rat in an individual cage between 0800 and 1200 by connecting this catheter to a Statham P23-ID pressure transducer. An electric R-C filter with a 2-s time constant was used to determine the mean arterial pressure, which was sampled for each individual animal, using an analog-digital converter (MacLab), at a rate of 4 samples/s for 1 h. The data were then averaged over the hour and presented as individual data. At the end of the experiment (day 10 of 1.5 M NaCl infusion), body weight was measured and an arterial blood sample (1 ml) was taken to measure plasma Na⁺, K⁺, and Cl⁻ concentration and the hematocrit. Rats were anesthetized by ether and the position of the catheter was confirmed by infusion of methylene blue into the catheter.

All of values presented are means ± SE. Data in Table 1 were analyzed by three-way ANOVA, with the strain (DS vs. DR), concentration of infusion solution (0.15 vs. 1.5 M), and infusion route (PV vs. IVC) as factors; data in Table 2 were analyzed by two-way ANOVA, with strain and infusion route as factors; and data in Fig. 3 were analyzed by three-way ANOVA, with period (light vs. dark), infusion route, and concentration of infusion solution as factors. The data in Figs. 4 and 5 were analyzed by repeated-measures ANOVA. The significance of post hoc comparisons was set at P < 0.05.

**RESULTS**

Table 1 shows overall daily water and saline consumption, as measured by volume. The data are presented as a 5-day average during 0.15 M NaCl infusion and a 7-day average during 1.5 M NaCl infusion. No difference in water consumption was seen during either infusion solution when comparing the PV and IVC routes [F(1,56) = 0.05, P = 0.83] or rat strain used [F(1,56) = 0.32, P = 0.57]. However, water consumption was significantly increased in both rat strains and by either infusion route during 1.5 M NaCl infusion compared with 0.15 M NaCl infusion [F(1,56) = 294.44, P < 0.0001]. In terms of saline consumption, a significant difference was seen with strain [F(1,56) = 8.36, P < 0.01] but not with either infusion solution concentration (P = 0.68) or infusion route (P = 0.15). In calculated percent saline consumption [saline consumption/(saline consumption + water consumption) × 100], significant effects were seen both with strain [F(1,56) = 8.17, P < 0.01] and infusion solution concentration [F(1,56) = 35.95, P < 0.0001].

**Table 1. Average daily water and saline consumption following PV and IVC infusion of 0.15 and 1.5 M NaCl**

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<tr>
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<th>Dahl Salt-Sensitive Rats</th>
<th>Dahl Salt-Resistant Rats</th>
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<tr>
<td></td>
<td>PV</td>
<td>IVC</td>
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<tr>
<td>Water consumption, ml/day</td>
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<tr>
<td>0.15 M NaCl infusion</td>
<td>4.9 ± 1.0</td>
<td>7.8 ± 1.4</td>
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<tr>
<td>1.5 M NaCl infusion</td>
<td>36.5 ± 2.3*</td>
<td>38.5 ± 5.2*</td>
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<tr>
<td>Saline consumption, ml/day</td>
<td></td>
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<tr>
<td>0.15 M NaCl infusion</td>
<td>11.7 ± 1.4†</td>
<td>10.6 ± 3.2</td>
</tr>
<tr>
<td>1.5 M NaCl infusion</td>
<td>9.6 ± 2.1†</td>
<td>16.7 ± 6.9</td>
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<tr>
<td>%Saline consumption</td>
<td></td>
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<tr>
<td>0.15 M NaCl infusion</td>
<td>67.0 ± 5.3†</td>
<td>49.5 ± 10.8</td>
</tr>
<tr>
<td>1.5 M NaCl infusion</td>
<td>19.4 ± 3.5†</td>
<td>26.8 ± 9.1†</td>
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</table>

Values are means ± SE; n = 8 animals for all groups. PV, portal vein; IVC, inferior vena cava. *P < 0.05, significantly different from 0.15 M NaCl infusion. †P < 0.05, significantly different from Dahl salt-resistant rats.
NaCl infusion, but this was lost during 1.5 M NaCl infusion. Irrespective of strain and infusion route, water consumption immediately increased on 1.5 M NaCl infusion, whereas saline consumption was not immediately decreased by 1.5 M NaCl infusion; in fact, during the light period, it tended to increase, whereas, during the dark period, it did not change for the first 2 days after the infusion solution was changed, then tended to decrease in the PV groups. The averaged data of 5 days during 0.15 M NaCl infusion and 7 days during 1.5 M NaCl infusion are shown in Fig. 3. Irrespective of strain and infusion route, water consumption was significantly increased during 1.5 M NaCl infusion ($P < 0.0001$), whereas the light-dark rhythm was unaffected, consumption always being significantly higher in the dark period. During 0.15 M NaCl infusion, saline consumption in the dark period was significantly higher than during the light period; however, this rhythm was abolished during 1.5 M NaCl infusion. In both strains, a significant interaction between period and infusion solution was seen in terms of saline consumption [DS rats: $F(1,56) = 12.16, P < 0.01$, DR rats: $F(1,56) = 5.38, P < 0.05$]. In both strains, saline consumption in the PV group during the dark period was significantly depressed by 1.5 M NaCl infusion; this effect was not seen in the IVC group.
Furthermore, irrespective of infusion route, 1.5 M NaCl infusion resulted in increased saline consumption during the light period in DS rats, but not in DR rats.

Figure 4 shows urinary Na\(^+\) excretion, Na\(^+\) balance, and fluid balance in DS rats. A significant interaction was seen between day and infusion route for urinary Na\(^+\) excretion \([F(11,154) = 2.15, P < 0.05]\), Na\(^+\) balance \([F(11,154) = 3.56, P < 0.001]\), cumulative Na\(^+\) balance \([F(11,154) = 10.12, P < 0.0001]\), fluid balance \([F(11,154) = 2.16, P < 0.05]\), and cumulative fluid balance \([F(11,154) = 7.00, P < 0.0001]\). No difference in urinary Na\(^+\) excretion, Na\(^+\) balance, and fluid balance was seen between the PV and IVC routes during 0.15 M NaCl infusion; however, when the infusion solution was changed to 1.5 M NaCl, lower values for urinary Na\(^+\) excretion and higher values for Na\(^+\) and fluid balance were seen in the IVC group compared with the PV group during the first 2 days of 1.5 M NaCl infusion; the values then reached to those of the PV group. These effects were less obvious in DR rats (Fig. 5). A significant interaction between day and infusion route was seen only for Na\(^+\) balance \([F(1,154) = 2.40, P < 0.01]\) and cumulative Na\(^+\) balance \([F(1,154) = 11.98, P < 0.0001]\), but not for urinary Na\(^+\) excretion \((P = 0.054)\), fluid balance \((P = 0.78)\), or cumulative fluid balance \((P = 0.90)\).

The values for arterial pressure, body weight increase, hematocrit, and plasma electrolyte concentrations are summarized in Table 2. Before infusion, the systolic arterial pressure was the same in all groups. However, at the end of the infusion experiment, the ANOVA results showed a significant effect of strain \([F(1,28) = 160.15, P < 0.0001]\) and infusion route \([F(1,28) = 15.81, P < 0.001]\) on mean arterial pressure. In DS rats, the mean arterial pressure in IVC group was significantly higher than that in the PV group \((P < 0.05)\), whereas no such difference was seen in DR rats \((P = 0.28)\). The body weight increase tended to be greater in DS rats, but the difference was not significant by ANOVA \([F(1,28) = 3.55, P = 0.07]\). No significant difference was found between groups in terms of the values for the hematocrit or plasma concentrations of Na\(^+\), K\(^+\), or Cl\(^-\).

DISCUSSION

The major findings of the present study are as follows. 1) In the PV groups, DS rats consumed more
saline than DR rats. 2) Regardless of the strain, hypertonic NaCl infusion caused a decrease in saline consumption in the dark period in the PV groups but not the IVC groups. 3) In DS rats, the mean arterial pressure in the IVC group was significantly higher than that in PV group; this was not the case in DR rats. 4) During 1.5 M NaCl infusion, the IVC groups retained more Na$^+$ than did the PV groups.

Conflicting results have been reported regarding preference for NaCl solution in Dahl rats. Wolf et al. (31) reported that DS rats exhibit a significantly lower absolute intake of isotonic saline than do DR rats. However, Rowland and Fregly (26) reported that preference for NaCl solution varies as a function of concentration but does not differ between the strains. Furthermore, Ferrell et al. (6) demonstrated that preference for NaCl solution is influenced by dietary NaCl levels in the weaning diet; under a 0.4% NaCl diet, the preference increases with concentration up to 0.18 M, then decreases steeply as the concentration is further increased, and DS rats exhibit a greater preference than DR rats; this effect becomes more evident when rats are preexposed to an 8% NaCl diet. The authors attributed these differences to possible “genetic drift” over the years. In the present study, conditions were more complicated. Oral Na$^+$ intake from food pellets was very low (<0.15 mmol/day), whereas oral Na$^+$ intake due to saline consumption was 0.5–2.5 mmol/day. In addition, the rats were loaded with either 1.8 or 18 mmol/day of Na$^+$ by PV or IVC infusion during the 0.15 and 1.5 M NaCl infusion periods, respectively. Regardless of the infusion route, 1.5 M NaCl infusion significantly increased saline consumption during the light period in DS rats but not DR rats. Measurements of consumption volume also suggest a difference in saline preference between DS and DR rats. When the NaCl solution was infused into the IVC, no difference in saline consumption or percent saline consumption was seen between DS and DR rats. However, when the NaCl solution was infused into the PV, both saline consumption and percent saline consumption were significantly greater in DS rats, compared with DR rats. Thus, under the conditions of the present study, DS rats exhibited a greater preference for isotonic saline compared with DR rats.
Table 2. Comparison of SAP before PV or IVC infusion; MAP and hematocrit and plasma concentrations of Na⁺, K⁺, and Cl⁻ after infusion; and changes in body weight

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<tr>
<td></td>
<td>PV</td>
<td>IVC</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>123 ± 4</td>
<td>124 ± 3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>138 ± 1t</td>
<td>150 ± 3*†</td>
</tr>
<tr>
<td>ΔBody weight, g</td>
<td>44 ± 6</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>36 ± 1</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Plasma Na⁺, meq/l</td>
<td>147 ± 1</td>
<td>146 ± 1</td>
</tr>
<tr>
<td>Plasma K⁺, meq/l</td>
<td>36 ± 0.1</td>
<td>39 ± 0.1</td>
</tr>
<tr>
<td>Plasma Cl⁻, meq/l</td>
<td>106 ± 2</td>
<td>105 ± 2</td>
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Values are means ± SE; n = 8 for all groups. Δ, change. Systolic arterial pressure (SAP) was measured immediately before the first operation; mean arterial pressure (MAP), hematocrit, and plasma ion levels were measured on day 10 of 1.5 M NaCl infusion; and body weight was measured before the first operation and on day 10 of 1.5 M NaCl infusion. *P < 0.05, significantly different from PV; †P < 0.05, significantly different from Dahl salt-resistant group.

There are some positive reports to support the involvement of the liver in the control of saline drinking behavior (14, 29, 30), despite a study to the contrary (7). Lin and Blake (14) first reported the positive results. They showed that PV infusion of 2 M NaCl into dehydrated rats decreases the short-term drinking of 0.15 M NaCl solution, whereas water intake is not affected, and that infusion of 2 M NaCl into the IVC, or of equiosmolar Na⁺-free solution into the PV, has no effect on the drinking of 0.15 M NaCl. However, Frankmann and Smith (7) found no difference in 0.3 M NaCl drinking between sham- and hepatic vagotomized (severing the hepatic branch from the right abdominal vagus nerve) rats under the conditions of Na⁺ depletion and gastric preload of NaCl. The discrepancy of the results may be partly due to differences in experimental conditions, i.e., NaCl concentration of the test solutions and the strains of rats used, etc. Furthermore, in the latter experiments, the hepatic denervation might be incomplete because of the following reasons. First, they did not sever the hepatic branch from the left abdominal vagus nerve, although the rat liver is innervated by both the right and left hepatic nerves, which leave from the right and left abdominal vagus nerve, respectively (15). Second, it is still unclear whether the hepatoportal Na⁺-receptive signals are mediated solely via the hepatic vagal nerve. Our previous report does not support this (19). We demonstrated that intravenous infusion of hypertonic NaCl decreases renal nerve activity, which is mediated by sinoarticular and cardiopulmonary baroreceptors and hepatic receptors. This decrease in renal nerve activity is not completely abolished by sinoarticular baroreceptor denervation and cervical vagotomy (involving the hepatic vagus nerve) but is completely abolished by sinoarticular baroreceptor denervation and cervical vagotomy plus total hepatic denervation.

In the present study, there was a tendency for a greater daily saline consumption during 1.5 M NaCl infusion in the IVC groups compared with the PV groups, although no significant difference was detectable by ANOVA. However, when the data for the light and dark periods were analyzed individually, PV infusion of 1.5 M NaCl significantly decreased saline consumption in the dark period in both strains; this effect was not seen in the IVC groups. These results support the idea that the hepatoportal Na⁺-sensitive mechanism plays a significant role in regulation of long-term saline drinking behavior.

If rats were fed normal salt diet (0.4% NaCl), they consumed ~1 mmol/day NaCl. Thus the physiological need for NaCl might be minimum during 1.5 M NaCl infusion (18 mmol/day NaCl load). However, the volume of saline consumed was not significantly decreased by 1.5 M NaCl infusion. It is known that rats have a hedonic liking for low concentration of salt that is unrelated to physiological need (2, 24) and it is therefore possible that the saline drinking behavior during 1.5 M NaCl infusion was induced not by a physiological need for NaCl but by this specific preference for saline solution. This raised a basic question, namely why 1.5 M NaCl infusion into the PV only depresses saline consumption during the dark period. In this context it is interesting to note that Box et al. (3) demonstrated, in rats, that a highly preferred solution appears to have a strong circadian component and is accentuated during the dark phase; this may partially explain the phase-related effect of PV 1.5 M NaCl infusion on saline drinking behavior.

In a preliminary study (our unpublished data), when DS rats were fed a high-NaCl (8%) diet for 10 days, the average daily Na⁺ intake was ~20 mmol and the mean arterial pressure increased to 136 ± 2 mmHg. In the present study, the mean arterial pressure in the DS/PV group (18 mmol/day NaCl load + saline drink) increased to 138 ± 1 mmHg, similar to the value seen in our preliminary study of oral NaCl load. The mean arterial pressure in the DS/IVC group (150 ± 3 mmHg) was significantly higher than that in DS/PV group. These data suggest that, in DS rats, the postabsorptive mechanism, possibly located in the liver, plays a significant role in controlling arterial pressure under conditions of Na⁺ overload.

The mechanisms involved in this increase in arterial pressure seen in DS rats are still unclear. Several candidates have been proposed, namely renal dysfunction (5), increased sympathetic nerve activity (27), and increased activity of Na⁺-K⁺-ATPase inhibitors (10). Na⁺ and fluid retention might be required for these mechanisms to operate or to induce hypertension. Greene et al. (9) demonstrated that intravenous Na⁺ load increases the blood volume in both DS and DR rats; however, an increase in arterial pressure is only seen in DS rats and is completely abolished if the blood volume was maintained constant. These results suggest that the significantly higher mean arterial pressure seen in the DS/PV group is due to significantly more retention of Na⁺ and fluid. In fact, the balance experiment in the present study demonstrated greater Na⁺ and fluid retention in the DS/PV group than in the DS/PV group. These results are consistent with our previous reports.
in which we demonstrated that a high-NaCl diet decreases renal nerve activity via the hepatic afferent nerves and this decrease plays a significant role in a postprandial natriuresis (18). If this mechanism is impaired by hepatic or renal denervation or by liver cirrhosis, the postprandial natriuresis is significantly attenuated (16, 18). In rats with liver cirrhosis, sensitivity of the hepatoportal Na+-sensitive mechanism is decreased and more Na+ is retained on a high-NaCl diet, but not on a normal-NaCl diet (28).

In the present study, the increased Na+ retention induced by the 1.5 M NaCl infusion in the IVC group was more obvious in DS rats compared with DR rats. This effect was only apparent for 2 days, but this positive balance was not compensated for in the following days. Furthermore, this effect was accompanied by a significantly greater retention of fluid in DS rats, but not in DR rats. The mechanism responsible for this difference between DS and DR rats is not apparent from the present data. One possible mechanism is that the sensitivity of the postabsorptive mechanism might be different between strains. Further studies will be required to determine this.

In conclusion, the postabsorptive mechanism, probably the hepatoportal Na+-sensitive mechanism, plays a significant role in the long-term control of saline drinking behavior, Na+ balance, and arterial pressure in DS rats. These effects are less evident in DR rats.

Perspectives

Maintenance of Na+ homeostasis is generally explained by the negative feedback system, including baroreceptor, volume receptor, and central osmoreceptor reflexes. Is this negative feedback system “ideal” for Na+ homeostasis? A negative feedback system initiates its operation in response to altered variables. The ideal control system would be based on prospective or negative feedforward control, which senses a disturbance, predicts the changes that will be caused by this disturbance, and directs the effector organs to cancel out the change. Thus changes in variables would be minimum. However, control error can occur, because this system operates on the basis of a prediction; this would be corrected by a negative feedback system.

Na+ consumed orally are absorbed from the intestine into the blood and circulate in the hepatic vasculature first, then go into the systemic circulation. The hepatoportal Na+-sensitive mechanism is triggered by the increase in portal venous Na+ concentration in advance of changes in systemic blood Na+ concentration and reflexively controls Na+ appetite, Na+ absorption, and Na+ excretion. The other important feature of this mechanism is that the portal venous blood flow is ~20% of the systemic blood flow. Thus the hepatoportal Na+-sensitive mechanism can detect amplified Na+ changes, whereas once absorbed Na+ enters into the systemic circulation, it is diluted by five times. Therefore, the hepatoportal Na+-sensitive mechanism may predict changes in systemic blood Na+ concentration on the basis of amplified changes in portal venous Na+ concentration, then control body fluid Na+ homeostasis in a prospective manner.

The authors would like to thank the late professor Hiroshi Hosomi for his suggestion of a concept of prospective control system; he died by asthma attack on February 19, 1996. This study was partly supported by a research grant from the Ministry of Education, Science and Culture of Japan.

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Received 14 July 1997; accepted in final form 29 December 1997.

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