REGULATION OF PLASMA ATRIAL NATRIURETIC PEPTIDE AND THE CARDIOPULMONARY BAROREFLEX IN THE RAT

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To study the physiological regulation of atrial natriuretic peptide (ANP), we examined the effects of volume expansion and depletion and the influence of cardiopulmonary baroreflex on plasma ANP levels in pentobarbital-anesthetized male Wistar rats.

The volume expansion by acute intravenous saline infusion (2% of body weight) increased central venous pressure (CVP) and decreased heart rate (HR) in rats with intact baroreflex. The plasma ANP level in the volume expanded group was significantly higher than that in the control rats (453 ± 100 vs 170 ± 40 pg/ml, p < 0.01). Conversely the plasma ANP level decreased from 214 ± 15 to 125 ± 13 pg/ml (p < 0.01) accompanied by a fall in CVP and an increase in HR after nonhypotensive hemorrhage (0.8% of body weight). Hypotensive hemorrhage (2% of body weight) caused a progressive decrease in CVP while plasma ANP did not decrease further (141 ± 25 pg/ml). Bilateral vagotomy did not modify either the basal plasma ANP level or the plasma ANP responses to volume expansion and depletion, though it inhibited HR response.

These results indicate that in the rat, plasma ANP responds not only to volume expansion but also to moderate volume depletion suggesting that ANP may have a physiological role in body fluid homeostasis. The ANP response to changes in blood volume appears to be independent of the cardiopulmonary baroreflex with vagal afferent.

Atrial natriuretic peptide (ANP) may play important roles in cardiovascular and body fluid regulation through its potent diuretic, natriuretic and vasorelaxant actions. It is now known that an increase in the plasma ANP level can be brought about by stimuli which cause atrial stretch such as volume expansion, high salt intake, water immersion, congestive heart failure and vasoconstrictor agents. But less is known about its response to the converse stimuli which decrease atrial pressure, although it may decrease plasma ANP.

The precise mechanisms regulating plasma ANP level remain unknown although mechanical force may act directly on the heart since atrial stretch of the isolated heart releases ANP. The cardiopulmonary baroreflex with vagal afferent influences blood pressure and body fluid homeostasis via the sympathetic nervous system, the renin-angiotensin system and vasopressin. Although a few reports suggest that the cardiopulmonary baroreflex has no role in ANP release.

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by volume loading it is not known whether cardiopulmonary baroreflex influences ANP regulation in the case of volume depletion.

In this study, plasma ANP concentrations were measured after volume expansion with acute saline infusion and after stepped volume depletion by nonhypotensive and hypotensive hemorrhage in the rat. We also investigated the influence of cardiopulmonary baroreflex on plasma ANP responses to volume stimuli by means of bilateral cervical vagotomy.

**METHODS**

Thirty-eight male Wistar rats weighing 300–400g each were examined under sodium pentobarbital anesthesia (50 mg/kg, i.p.). Catheters made from PE-50 polyethylene tube were inserted in the carotid artery, superior vena cava, and a femoral vein. Arterial blood pressure and central venous pressure (CVP) were monitored continuously using Statham P23ID transducers (Gould, USA) and a multichannel recorder (Hewlett Packard 7758B, USA). Heart rate (HR) was calculated from the arterial pressure wave.

Seven rats were infused with isotonic saline in an amount that was 2% of body weight via the femoral vein over 1 minute. Blood samples were obtained by decapitation 3 minutes after the injection. Another 7 rats were examined without saline infusion (control group).

Stepped hemorrhage was carried out in 5 rats. First, a volume of blood equaling 0.8% of body weight was withdrawn through the arterial catheter within 3 minutes. This blood was used to measure basal plasma ANP level. Five minutes later, an additional volume of blood equaling 1.2% of body weight was withdrawn in the same manner and it was used as the blood sample after nonhypotensive hemorrhage. Finally, a third blood sample was obtained to measure the plasma ANP level after the hypotensive hemorrhage.

Bilateral cervical vagotomy was carried out in another 19 rats. These rats were given either the

| TABLE 1 | MEAN BLOOD PRESSURE, CENTRAL VENOUS PRESSURE AND HEART RATE BEFORE AND AFTER VOLUME EXPANSION WITH INTRAVENOUS ISOTONIC SALINE (2% OF BODY WEIGHT) IN THE INTACT AND VAGOTOMIZED RATS |
|-------------------------|-----------------|-----------------|-----------------|
|                         | MBP (mmHg)     | CVP (mmHg)      | HR (beats/min)  |
|                         | bef  aft        | bef  aft        | bef  aft        |
| Intact vagi             |                |                 |                 |
| exp (−) (n = 7)         | 114 ± 9        | 4.8 ± 0.6       | 344 ± 15        |
| exp (+) (n = 7)         | 117 ± 5        | 108 ± 5**       | 9.0 ± 1.0***    |
| Vagotomized             |                |                 |                 |
| exp (−) (n = 7)         | 120 ± 3        | 4.2 ± 0.5       | 379 ± 9         |
| exp (+) (n = 7)         | 130 ± 6        | 122 ± 5*        | 5.1 ± 0.5       |

Results are the means ± SE. *p < 0.05, **p < 0.01, ***p < 0.001 for difference between before (bef) and after (aft) volume expansion. MBP = mean blood pressure; CVP = central venous pressure; HR = heart rate; exp (−) = without volume expansion; exp (+) = with volume expansion. Baseline MBP, CVP and HR values were not significantly different between the exp (−) and exp (+) groups.

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same amount of saline infusion (n = 7) or no infusion (n = 7), or the stepped hemorrhage (n = 5).

The blood samples were collected in chilled tubes and immediately centrifuged at 4°C. Each plasma aliquot was stored at -20°C until the assay. The ANP concentration was determined by radioimmunoassay after SEP-PAK extraction. We used rabbit anti-α human ANP serum (Peninsula Lab., USA; RAS-8798), which showed 100% cross-reactivity with α human ANP, α rat ANP, and rat atriopeptin III. The detail of the assay procedure was described previously.12

The data were expressed as the mean ± standard error. They were analyzed using paired and non-paired Student's t test. P values of less than 5% were considered statistically significant.

### RESULTS

Volume expansion with isotonic saline equaling 2% of body weight caused a transient increase in CVP, a small decrease in mean blood pressure (MBP) and a reduction in heart rate in the 7 rats (Table I). These levels were stable during the experimental procedure in the control group. The plasma ANP level was significantly higher in the saline infusion group than in the control group (453 ± 100 vs 170 ± 40 pg/ml, p < 0.05, Fig. 1).

In the vagotomized group, baseline MBP and HR tended to be higher than those in the rats with intact vagi, although the difference was not significant (Table I). There was also no difference in the basal levels of CVP and plasma ANP between the groups. The saline infusion produced similar CVP and MBP changes but less HR response in the vagotomized group than in the intact group. Plasma ANP increased significantly with the saline infusion (465 ± 121 vs 104 ± 43 pg/ml, p < 0.05), and these levels did not differ from those in the rats with intact vagi (Fig. 1).

After hemorrhage of 0.8% of the body weight in 5 rats, CVP decreased and HR increased while MBP did not change significantly (Table II). The plasma ANP concentration decreased from 214 ± 15 to 125 ± 13 pg/ml (p < 0.01, Fig. 2). After hemorrhage of 2% of the body weight, CVP decreased further, MBP fell markedly, and HR tended to decrease. The level of plasma ANP was similar to that after nonhypotensive hemorrhage (141 ± 25 pg/ml).

In the 5 vagotomized rats, the stepped hemorrhage caused similar CVP change, slightly greater

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**TABLE II** MEAN BLOOD PRESSURE, CENTRAL VENOUS PRESSURE AND HEART RATE BEFORE AND AFTER STEPPED HEMORRHAGE IN INTACT AND VAGOTOMIZED RATS

<table>
<thead>
<tr>
<th></th>
<th>MBP (mmHg)</th>
<th>CVP (mmHg)</th>
<th>HR (beats/min)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Intact</td>
<td></td>
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<tr>
<td>vagi (n = 5)</td>
<td>118 ± 3</td>
<td>114 ± 3</td>
<td>62 ± 2***</td>
</tr>
<tr>
<td>Vagotomized</td>
<td>118 ± 6</td>
<td>104 ± 7*</td>
<td>67 ± 8***</td>
</tr>
</tbody>
</table>

Results are the means ± SE. *p < 0.05, ***p < 0.001 for difference between either 1 and C or 2 and C.

MBP = mean blood pressure; CVP = central venous pressure; HR = heart rate; C = before hemorrhage; 1 = hemorrhage of 0.8% of body weight; 2 = hemorrhage of 2% of body weight.

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MBP reduction and markedly blunted HR response compared with the intact group (Table II). Plasma ANP responses to the stepped hemorrhage in the vagotomized group were similar to those in the non-vagotomized group (Fig. 2). Therefore, the response of plasma ANP to volume expansion and depletion was not modified by bilateral vagotomy.

DISCUSSION

This study showed that the plasma ANP level increased in response to volume expansion by acute saline infusion and decreased in response to moderate volume depletion by nonhypotensive hemorrhage in the rat. Many investigators have reported increases in plasma ANP by volume overload in the rat, the dog, and man, while the response of ANP to volume depletion is less known. We employed the stepped hemorrhage method since it has been used to examine the influence of blood volume reduction on neurohormonal factors such as the sympathetic nervous system, the renin-angiotensin system and vasopressin. Our results agreed with the observation of Sagnella et al. who reported a decrease in plasma ANP level in man when dietary sodium intake was reduced. Oghihara et al. reported that whole-body tilting caused a transient decrease in plasma ANP and passive leg raising increased ANP in man. Taken together, the changes in plasma ANP by both volume expansion and depletion appear to act to maintain body fluid homeostasis, suggesting that ANP is an important physiological regulator of body fluid.

Hypotensive hemorrhage did not result in a larger decrease in plasma ANP than nonhypotensive hemorrhage although the reduction in CVP was greater in this study. The reason for this is not clear, but the nonhypotensive hemorrhage might be a maximal stimulation for the decrease of plasma ANP. On the other hand, it is known that the hypotensive hemorrhage causes massive secretion of vasopressin and activates the sympathetic nervous system and the renin-angiotensin system. These pressor systems may interfere with further reduction in plasma ANP induced by hypotensive hemorrhage since they stimulate ANP release.

In vagotomized rats, there was marked attenuation of HR response to volume load or depletion, suggesting effective blockade of the cardiopulmonary baroreflex. However, bilateral vagotomy modified neither the basal level of plasma ANP nor its changes caused by the volume stimuli. The results indicate that cardiopulmonary baroreflex with vagal afferent does not play a role in plasma ANP response to either volume expansion or depletion. Since Lang et al. demonstrated that ANP could be released in response to atrial pressure load in isolated heart preparations, it appears that the atrial stretch itself triggers ANP release within the heart. Our study confirmed a few previous reports. Ledesme et al. observed that bilateral cervical vagotomy did not prevent the increase in plasma ANP due to mitral obstruction in the dog. Eskay et al. reported that vagotomy did not alter ANP release by volume loading in rats. However, they observed that cardiac denervation in pithed rats blocked ANP release, suggesting neural influence on ANP regulation.

It is well known that cardiopulmonary baroreceptor reflex with vagal afferent plays an important role in body fluid homeostasis through the sympathetic nervous system, the renin-angiotensin system and vasopressin. The relative significance of the cardiopulmonary baroreflex and ANP in body fluid regulation in response to acute volume changes is not understood yet. It was reported that bilateral vagotomy blunted the increase in urine volume and natriuresis produced by acute volume expansion in the dog. On the other hand, Schwab et al. observed that right atrial appendectomy in the rat attenuated volume expansion-induced increase in plasma ANP and urinary sodium excretion. The lack of dependency of ANP release on the cardiopulmonary baroreflex may function as an important component in the multifactorial regulatory system of body fluid balance and cardiovascular function.

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