Physiological Factors of Atrial Natriuretic Polypeptide Release and Its Neural Regulation in Conscious Dogs

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We have examined physiological factors in atrial natriuretic polypeptide (ANP) release and whether or not the cardiac nerves control release of ANP. Two possible factors were tested, an increase in plasma sodium level (PNa) and an increase in atrial pressure. Injection of 1.0 or 2.0 mEq/kg of sodium ions elevated PNa by 5.3 ± 0.3 or 7.3 ± 0.4 mEq/L, respectively, but plasma ANP level (PANP) did not change. Infusion of 18 ml/kg of 3% Dextran-40 over 5 min increased mean left atrial pressure (MLAP) by 7.6 ± 0.9 mmHg. PANP increased from 206 ± 17 pg/ml to 260 ± 25 pg/ml, which was not significant. PANP, corrected for hemodilution, significantly increased to 348 ± 34 pg/ml. These results suggest that PNa increase does not promote ANP release, but that an atrial pressure increase does. This transient volume load did not induce full response of the ANP releasing system. A prolonged volume load for 45 min increased corrected PANP to 435 ± 73 pg/ml. A close linear correlation was found between the increases in MLAP and PANP. These facts indicate that prolonged volume expansion is necessary to induce full response of the ANP releasing system. Complete cardiac denervation did not affect the tonic level of plasma ANP, volume expansion-induced increase in PANP, or the sensitivity of the ANP releasing system. Thus we conclude that the cardiac nerves do not control ANP release caused by volume expansion.

Atrial natriuretic polypeptide (ANP) has been reported to have a specific and potent natriuretic activity.1–4 Tanaka et al5 and Shenker et al6 have demonstrated that high sodium intake induces an increase in plasma ANP concentration. These facts indicate that ANP may play a controlling role in maintaining plasma sodium concentration (PNa) or extracellular fluid volume. Based on control theory, we hypothesized two possible promoters of ANP release, an increase in plasma sodium concentration (PNa) and an increase in blood volume. In the present study, we examined the effects of rapid sodium load and acute volume load on ANP secretion in conscious dogs. Recently Eskay et al demonstrated that cardiac denervation in a pithed rat preparation blocked release of ANP.7 Currie et al8 and Sonnenberg9 reported that stimulation of alpha-adrenergic receptors induced ANP release from the isolated rat heart and from

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METHODS

Twenty four mongrel dogs (9–18 kg body weight) were divided into 2 groups: a cardiac nerve intact (INT) group (13 dogs) and a cardiac denervated (CD) group (11 dogs). Dogs of the INT group were anesthetized with an intravenous injection of pentobarbital sodium, intubated, and placed on a respirator. Polyethylene catheters were aseptically placed in the aorta via the left superficial cervical artery and in the superior vena cava via the left external jugular vein. A left thoracotomy was performed to implant polyethylene catheters in the left atrium and the descending aorta. The catheters were exteriorized at the back and filled with heparin sodium solution (500 units/ml).

Cardiac denervation was performed on 11 dogs of the CD group following modified Randall's method.12 Cardiac denervation was confirmed after the dog had recovered from surgery as described in details by Wang et al.13

Experiments were conducted in conscious dogs at least 2 weeks after the operation. Each dog was fasted but had free access to water during the 12 hours prior to the experiment. On the day of the experiment, the dogs were laid quietly on a table. Pressure transducers were connected to a superficial cervical arterial catheter and a left atrial catheter for systemic arterial pressure and left atrial pressure measurements, respectively. The mean arterial pressure (MAP) and mean left atrial pressure (MLAP) were obtained using a low-pass filter with a 2-sec time constant. Heart rate (HR) was derived from the pressure pulse interval via a cardiotachometer. After MAP, HR, and MLAP stabilized, one of 4 protocols was begun: 1) rapid sodium load in an INT dog (n = 8); 2) transient (n = 6) or prolonged (n = 7) volume expansion in an INT dog; 3) measurement of basal level of plasma ANP before and after cardiac denervation in a CD dog (n = 8); 4) prolonged volume expansion in a CD dog (n = 6).

Plasma ANP was assayed by a direct radioimmunoassay without prior extraction of ANP, using a specific antiserum to synthetic α-human ANP. Details of the radioimmunoassay procedure have been described previously.14 In order to estimate the amount of ANP secreted into circulation, assayed plasma ANP concentration was corrected for hemodilution due to massive fluid infusion. The plasma ANP level was corrected as follow:

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Corrected ANP level = Assayed ANP level \times (H_1(100 - H_2) / H_2(100 - H_1)) where H_1 is the mean value of hematocrits (Hts) before the infusion, and H_2 is the value of Ht at a given time after the start infusion.

Plasma sodium concentrations were measured by standard flame photometry (Flame Photometer 750, Hitachi). Plasma osmolality was measured by freezing point depression (Osmometer OM801, Vogel, Giessen, Germany). Ht was measured by the capillary method.

Data from each experiment were analyzed by two-way analysis of variance for repeated measurements on the same valuable. When statistically significant differences were detected, Dunnett's multiple testing was applied to test for significant differences between the mean of control values and the values at a given time after rapid sodium load or volume load. Student's t-test was used to identify significant differences between the two groups. A p-value of less than 0.05 was taken as statistically significant. Values are expressed as means ± SE.

RESULTS AND DISCUSSIONS

Rapid sodium load
To stimulate the ANP releasing system with an increase in plasma Na level, NaCl solution in small volume and at high concentration was injected into 8 INT dogs. As shown in Fig. 1, plasma Na concentration increased by 5.1 ±
sodium (2 mEq/kg b. w.) into 5 INT dogs (data not shown). The increase in plasma Na level (7.3 ± 0.5 mEq/L) after injection of 2 mEq/kg b. w. was significantly higher than that after 1 mEq/kg b. w. However, PANP still did not change. Release of arginine vasopressin\textsuperscript{15} or aldosterone\textsuperscript{16} has been reported to be affected by a change of 3 to 4 mEq/L in plasma Na level, which is smaller than that of the present study. Therefore, our sodium load was large enough to perturb the endocrine system. Based on these results, we speculate that an increase in plasma Na level may not be a physiological stimulus to the ANP releasing system.

**Acute volume load**

When 18 ml/kg of isosmotic and isoosmotic fluid (about 20% of estimated blood volume) was infused over 5 min into 6 INT dogs, MLAP increased by 7.6 ± 0.9 and PANP also increased slightly (Fig. 2-A). The marked decrease in Ht indicates that acute massive infusion caused hemodilution. PANP corrected for hemodilution increased significantly. Thus acute volume load stimulates ANP release. These results are supported by the reports of Lang et al\textsuperscript{11} and Ledsome et al\textsuperscript{10} However, this transient volume load is not sufficient to induce full response of the ANP releasing system. Fig. 2-A shows that the peak time of corrected PANP lagged 3 to 5 min behind that of MLAP. When the corrected PANP reached its peak level, MLAP had already started to decrease. These facts mean that the stimulus to the ANP releasing system was reduced before the ANP releasing system fully responded. So a prolonged volume load experiment was performed (Fig. 2-B). During the supplemental infusion period, a greater increase in corrected and uncorrected PANP than in the transient load experiment was noted. The peak time of PANP lag get about 10 min behind the the maximum increase in MLAP. After PANP reached its maximum level it did not change significantly until the end of supplemental infusion period. A close linear correlation was found between increases in MLAP and corrected PANP (r = 0.91). These results indicate that this prolonged volume load stimulated the ANP releasing system fully.

**Effects of cardiac denervation on tonic level of plasma ANP**

To determine whether or not cardiac nerves control the release of ANP, we examined the

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*Fig. 3. Effects of cardiac denervation on the increase in ANP release in response to prolonged volume expansion. 18 ml/kg b. w. of 3% Dextran-40 in saline was infused over 5 min, followed by supplemental infusion for 40 min. MLAP increased by 8.7 ± 1.3 mmHg in CD dogs, which was not significantly different from INT dogs. PANP corrected for hemodilution increased from 196 ± 18 pg/ml to 435 ± 73 pg/ml in INT dogs and from 191 ± 24 pg/ml to 462 ± 61 pg/ml in CD dogs at 15 min after the start of infusion. There was no significant difference in PANP increase between the INT group and the CD group. Data are expressed as mean ± SE. *p < 0.05 compared with control in each group.*

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0.3 mEq/L immediately after the injection. However, plasma ANP level (PANP) was not altered significantly by this sodium load. The decrease in Ht shown in Fig. 1 indicates that the administration of a large amount of NaCl caused hemodilution due to capillary fluid shift from the interstitial space. To estimate the amount of ANP released into circulation, assayed PANPs were corrected for hemodilution. PANP corrected for hemodilution was not either altered significantly. This sodium load of 1 mEq/kg b. w. might not be sufficient to accelerate ANP release. We also injected a double amount of
effects of intrapericardial cardiac denervation on circulating ANP. There was no significant difference between tonic levels of plasma ANP before and after cardiac denervation (within a week after). Fig. 3 shows that the control value of PANP in CD dogs was not significantly different from that in INT dogs. These values in the CD dogs were obtained more than 2 weeks after cardiac denervation. This suggests that the tonic secretion of ANP into circulation may not be regulated by the cardiac nerves.

Effects of cardiac denervation on prolonged volume load-induced ANP release

We examined the sensitivity of the ANP releasing system in CD dogs to prolonged volume load. The extent of the PANP increase in CD dogs was not significantly different from that in INT dogs. The time course of increase in CD dogs was not significantly different from that in INT dogs (Fig. 3). We next examined the relationship between changes in MLAP and PANP. A close linear correlation was found in both groups between changes in MLAP and normalized PANP corrected for hemodilution. The slope of the regression line in the CD group was not significantly different from that in the INT group. Thus the ratio of increase in PANP to increase in MLAP was not affected by cardiac denervation. This fact corresponds to an equal responsiveness of the ANP releasing system to changes in MLAP. These suggest that the cardiac nerves do not mediate ANP release due to changes in MLAP.

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