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The Effect of Angiotensin II and ADH on the Secretion of Atrial Natriuretic Factor (42678)

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Abstract. Studies in intact animals have suggested that angiotensin II (AII) and antidiuretic hormone (ADH) increase the plasma concentration of atrial natriuretic factor (ANF). The purpose of these studies was to examine the effects of AII and ADH on ANF secretion in a rat heart–lung preparation under conditions where aortic pressure could be regulated and other indirect effects of these hormones eliminated. ANF secretion was estimated as the total amount of ANF present in a perfusion reservoir at the end of each 30-min period. A pump was used to deliver a fluorocarbon perfusate to the right atrium at rates of either 2 or 5 ml/min. In a time control series where venous return was maintained at 2 ml/min for three 30-min periods ANF secretion was 672 ± 114, 794 ± 91, and 793 ± 125 pg/min (n = 6, $P > 0.05$). When venous return was increased from 2 to 5 ml/min ANF secretion increased from 669 ± 81 to 1089 ± 127 pg/min ($P < 0.01$). The addition of AII to the perfusate in concentrations of 50, 100, or 200 pg/ml (n = 6 in each group) had no significant effect on basal ANF secretion or the ANF response to increasing venous return. Similarly, the addition of ADH to the perfusate in concentrations of 5, 25, or 100 pg/ml had no significant effect on ANF release from the heart. These results suggest that the ability of AII and ADH to increase plasma ANF concentration *in vivo* may be due to the effects of these hormones on right or left atrial pressure.

Atrial natriuretic factor (ANF) is a substance which is secreted by the heart in response to distention of the atria (1–4) and it has been shown to produce dramatic increases in sodium execretion by the kidneys (5). It appears likely that ANF plays a major role in the natriuresis and diuresis that accompanies acute blood volume expansion (6–8). Atrial appendectomy in the rat has been shown to attenuate the increase in plasma ANF concentration that accompanies volume expansion (9) and also attenuates the diuretic and natriuretic responses (9, 10). Furthermore, antibodies directed against ANF attenuate the natriuresis and diuresis of blood volume expansion (11).

There is evidence to suggest that antidiuretic hormone (ADH) may increase ANF secretion (12–14), but it is not known if ADH stimulates ANF release directly or acts indirectly by producing changes in right or left atrial pressure. Katsube *et al.* (13) demonstrated that changes in plasma ANF in rats infused with vasopressin or angiotensin II correlated with changes in right atrial pressure. However, Sonnenberg and Veress (12) used a bioassay to show a stimulation of ANF release by vasopressin in cultured atrial tissue, which suggests a direct effect of ADH on ANF secretion.

The purpose of the present study was to determine if ADH and angiotensin II have direct effects on ANF secretion. Therefore, the effects of these hormones on ANF release were studied in a rat heart–lung preparation where fluid composition, venous return, and aortic pressure can be controlled and other neural and humoral influences could be eliminated.

Materials and Methods. The surgical preparation of the rat heart–lung preparation has been described previously (15). In brief, male Sprague–Dawley rats weighing 300 to 400 g were anesthetized with sodium pentobarbital (60 mg/kg). A tracheotomy was performed and the rats were placed on a respirator delivering 95% O_2, 5% CO_2. The chest was opened and a polyethylene catheter (PE-90) was placed in the innominate artery and advanced to its junction with the aorta. A second catheter was placed in the inferior vena cava. Loose ligatures were placed around the jugular veins and around the aorta just distal to the aortic catheter. The catheters in the inferior vena cava and aorta were connected to a reservoir containing a

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fluorocarbon-based perfusate which flowed by gravity into the right atrium (Oxypherol, Green Cross). A clamp in the aortic line was used to maintain aortic pressure at approximately 80 mm Hg throughout the study. Following ligation of the loose ligatures around the jugular veins and aorta the cardiopulmonary circulation was isolated and perfused *in situ*. During an initial stabilization period of 20 min the reservoir was set at a height of 2 cm above the heart and the perfusate flowed into the right atrium by gravity. Following this equilibration period a pump was used to return the perfusate to the right atrium at rates of either 2 or 5 ml/min. The pump was connected to a reservoir containing approximately 30 ml of fresh perfusate. The perfusate was changed again at the end of each 30-min period.

Time control series. Six heart–lung preparations were perfused for three 30-min periods with the atrial infusion rate maintained at 2 ml/min and aortic pressure set at 80 mm Hg. No drugs or hormones were added to the perfusate in this series. In the series described below, atrial stretch (increased venous return) was used as a mechanism of increasing ANF secretion. The purpose of this time control series was to demonstrate that ANF secretion in this preparation is stable when the atria are not stretched by increasing venous return.

Effect of ADH on ANF secretion. Heart–lung preparations were perfused for one 30-min period with the atrial infusion rate set at 2 ml/min and then the infusion rate was increased to 5 ml/min for a second 30-min period. Arginine vasopressin (Sigma Chemical Co., St. Louis, MO) was added to the perfusate in concentrations of 0, 5, 25, or 100 pg/ml. Six preparations were perfused with each hormone concentration.

Effect of angiotensin II on ANF secretion. The protocol for this series was identical to the one used for the ADH series above. Angiotensin II (Sigma Chemical Co., St. Louis, MO) was added to the perfusate in concentrations of 0, 50, 100, or 200 pg/ml ($n = 6$ in each group).

Sampling and assay for ANF. At the end of each 30-min perfusion period the exact volume of perfusate in the reservoir was obtained and a sample frozen for later analysis. The ANF content of the samples was measured by a radioimmunoassay (16) after removal of the fluorocarbon particles from the sample. This was accomplished by adding 200 μl of a 20% dextran solution to each 1-ml aliquot of perfusate and centrifugation.

The radioimmunoassay employed rat ANF-(99-126) as a standard (Chemical Dynamics Corp., 08-1552-00), rabbit antisera (Peninsula Laboratories, RAS 8798), and ^{125}I-labeled ANF-(99-126) as a tracer (Amersham, IM-186). Specific details on the assay have been given elsewhere (15). Evidence suggests that the 28 amino acid peptide termed rat ANF-(99-126) (17) is the primary form of ANF released by isolated perfused rat hearts (18).

ANF secretion rate for each perfusion period was estimated by multiplying the perfusate ANF concentration by the reservoir volume to obtain the total ANF secreted. The total ANF produced per time period was then divided by the length of the period to obtain hormone secretion rate.

The data were analyzed by either a paired *t* test or an analysis of variance (AOV) where appropriate. In all cases a P value of less than 0.05 was considered the criterion for statistical significance.

Results and Discussion. In the time control series, where six heart–lung preparations were perfused for three 30-min periods with a constant atrial infusion rate of 2 ml/min, ANF release averaged 672 ± 114, 794 ± 91, and 793 ± 125 pg/min. These values were not significantly different.

The results of the vasopressin series are shown in Fig. 1. In the first series where vasopressin was not added to the perfusate (zero dose) ANF secretion averaged 624 ± 81 pg/min at an infusion rate of 2 ml/min and increased to 963 pg/min at an infusion rate of 5 ml/min ($P < 0.05$). When vasopressin was added to the perfusate in concentrations of 5, 25, or 200 pg/ml ($n = 6$ in each), ANF secretion was not different from the first series (zero dose) at either infusion rate ($P > 0.05$, AOV).

The results of the angiotensin II series are shown in Fig. 2. This series of experiments was conducted in a fashion similar to the vasopressin series with the exception that concentrations of angiotensin II of 0, 50,

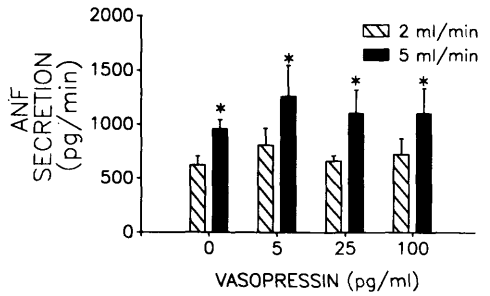

FIG. 1. The effect of arginine vasopressin on the release of atrial natriuretic factor (ANF) from a perfused rat heart–lung preparation. Preparations were perfused for one 30-min period with venous return set at 2 ml/min (hatched bars) and a second 30-min period at 5 ml/min (solid bars). Vasopressin was added to the perfusate in the concentrations indicated ($n = 6$ per group). Values are means + SEM. * $P < 0.05$ from first perfusion period.

100, and 200 pg/ml were used. In the first series (zero dose) ANF secretion averaged 669 ± 81 pg/min at an infusion rate of 2 ml/min and increased to 1089 ± 127 pg/min at an infusion rate of 5 ml/min ($P < 0.01$). Regardless of the concentration of angiotensin II in the perfusate there was no significant effect of angiotensin II on ANF secretion ($P > 0.05$, compared to the zero dose series, AOV).

The potencies of both vasopressin and angiotensin II stock solutions were verified by injecting aliquots of each into anesthetized rats and observing changes in arterial blood pressure. The hormones were dissolved in either saline or fluorocarbon perfusate prior to injection. The results of these tests showed that the hormones used in this study were biologically active and that dissolving them in the perfusate did not reduce their bioavailability.

Previous work from this laboratory using a rat heart–lung preparation showed that atrial stretch releases a natriuretic factor from the heart (1). These findings were subsequently confirmed in intact animals by others using radioimmunoassays (2–4). More recently, this laboratory (15) showed that both changes in venous return and changes in aortic pressure can alter the release of ANF from the heart–lung preparation. The findings support studies in intact animals showing that increased stretch of either the left or the right atrium could stimulate ANF release from the heart (4).

Several studies have suggested a hormonal control or modulation of ANF secretion by ADH or angiotensin II (12–14). The purpose of the present study was to determine if ADH or antiotensin II would effect ANF release from the heart in the isolated preparation where specific concentrations of the hormones could be added to the perfusate. Furthermore, in the rat heart–lung preparation, the atrial infusion rate could be regulated and aortic pressure maintained constant in each experiment.

The results showed that the concentrations of ADH (Fig. 1) and angiotensin II (Fig. 2) employed here had no significant effect on ANF secretion. Also, neither hormone had a significant effect on the ANF response to increasing venous return (Figs. 1 and 2).

It has been previously shown that ADH may have a direct stimulatory effect on ANF released from cultured atrial tissue where ANF was measured by a bioassay (12). Two laboratories have also reported increases in plasma ANF concentration in intact animals with ADH injections. Itoh *et al.* (14) found that large injections of ADH increased plasma ANF concentration in conscious rats. Smaller injections of ADH had no effect on basal ANF levels but augmented the ANF response to blood volume expansion. They did not report changes in arterial pressure or

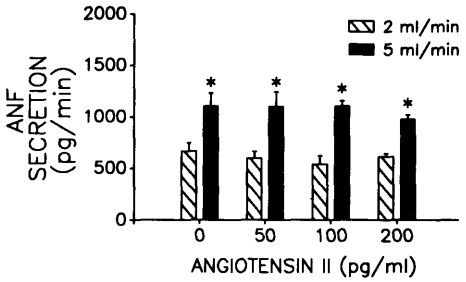

FIG. 2. The effect of angiotensin II on the release of atrial natriuretic factor (ANF) from a perfused rat heart–lung preparation. Preparations were perfused for one 30-min period with venous return set at 2 ml/min (hatched bars) and a second 30-min period at 5 ml/min (solid bars). Angiotensin II was added to the perfusate in the concentrations indicated ($n = 6$ per group). Values are means + SEM. * $P < 0.05$ from first perfusion period.

atrial pressures. Katsube *et al.* (13) showed that several vasoconstrictors, including ADH, angiotensin II, and phenylephrine, increased the plasma levels of immunoreactive atriopeptin III in anesthetized rats. They also reported (13) a very strong correlation between changes in right atrial pressure and changes in the plasma concentration of ANF when ADH and angiotensin II were infused. Their results suggest that ADH and angiotensin II increase ANF secretion indirectly through their effects on resistance and capacitance vessels which ultimately results in left or right atrial distention. The results of the present study in an isolated preparation support the suggestion that ADH and angiotensin II do not have direct stimulatory effects on the ANF-secreting cells.

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1. Dietz JR. Release of natriuretic factor from rat heart–lung preparation by atrial distention. Amer J Physiol **247**:R1093–R1096, 1984.
2. Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger T. Atrial natriuretic factor: A circulating hormone stimulated by volume loading. Nature (London) **314**:264–266, 1985.
3. Ledsome JR, Wilson N, Courneya CA, Rankin AJ. Release of atrial natriuretic peptide by atrial distention. Canad J Physiol Pharmacol **63**(6):739–742, 1985.
4. Metzler CH, Lee M, Thrasher TN, Ramsay DJ. Increased right or left atrial pressure stimulates release of atrial natriuretic peptides in conscious dogs. Endocrinology **119**(5):2396–2398, 1986.
5. DeBold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci **28**:89–94, 1981.
6. Verburg KM, Freeman RH, Davis JO, Villarreal D, Vari RC. Control of atrial natriuretic factor release in conscious dogs. Amer J Physiol **251**:R947–R956, 1986.
7. Ogihara T, Shima J, Hara H, Tabuchi Y, Hashizume K, Nagana M, Katahira K, Kangawa K, Matsuo H, Kumahara Y. Significant increase in plasma immunoreactive atrial natriuretic polypeptide concentration during head-out water immersion. Life Sci **38**:2413–2418, 1986.
8. Schwab TR, Edwards BS, Heublein DM, Burnett JC. Role of atrial natriuretic peptide in volume-expansion natriuresis. Amer J Physiol **251**:R310–R313, 1986.
9. Villarreal D, Freeman RH, Davis JO, Verburg KM, Vari RC. Effects of atrial appendectomy on circulating atrial natriuretic factor during volume expansion in the rat. Proc Soc Exp Biol Med **183**:54–58, 1986.
10. Veress AT, Sonnenberg H. Right atrial appendectomy reduces the renal response to acute hypervolemia in the rat. Amer J Physiol **247**:R610–R613, 1984.
11. Hirth C, Stasch JP, John A, Kazda S, Morich F, Neuser D, Wohlfeil S. The renal response to acute hypervolemia is caused by atrial natriuretic peptides. J Cardiovasc Pharmacol **8**(2):268–275, 1986.
12. Sonnenberg H, Veress AT. Cellular mechanism of release of atrial natriuretic factor. Biochem Biophys Res Commun **124**(2):443–449, 1984.
13. Katsube N, Schwartz D, Needleman P. Release of atriopeptin in the rat by vasoconstrictors or water immersion correlates with changes in right atrial pressure. Biochem Biophys Res Commun **133**(3):937–944, 1985.
14. Itoh H, Nakao K, Yamada T, Morii N, Shiono S, Sugawara A, Saito Y, Mukoyama M, Arai H, Katsuura G, Eigyo M, Matsushita A, Imura H. Modulatory role of vasopressin in secretion of atrial natriuretic polypeptide in conscious rats. Endocrinology **120**(5):2186–2188, 1987.
15. Dietz JR. Control of atrial natriuretic factor release from a rat heart–lung preparation. Amer J Physiol **252**:R498–R502, 1987.
16. Larose P, Meloche S, duSovich P, Delean A, Ong H. Radioimmunoassay of atrial natriuretic factor: Human plasma levels. Biochem Biophys Res Commun **130**:553–558, 1985.
17. Dzau VJ, Baxter JO, Cantin M, DeBold A, Ganten D, Gross K, Husain A, Inagami T, Menard J, Poole S, Robertson JIS, Tang J, Yamamoto K. Nomenclature for atrial peptides. N Engl J Med **316**(20):1278–1279, 1987.
18. Thibault G, Garcia R, Gutkowska J, Lazure C, Seidah NG, Chretien M, Genest J, Cantin M. Identification of the released form of atrial natriuretic factor by the perfused rat heart. Proc Soc Exp Biol Med **182**:137–141, 1986.

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