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A Potent Hypotensive Factor in Chicken Left Ventricle (42615)

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Abstract. Chicken artria and ventricles both have membrane-bound granules which resemble those containing atriopeptin (ANP) in mammals. However, nothing is known about the contents of the avian granules. A previous study in chickens showed that although extracts of *whole* chicken heart or synthetic rat ANP both caused profound hypotension, ANP caused both natriuresis and diuresis, while chicken heart extract did not. The present study sought to locate the region(s) of chicken heart containing the hypotensive activity, and to observe the effect on sodium and water excretion and blood pressure in rats. Acid extracts of either atrium, either ventricle, ventricular septum, skeletal muscle, and liver were identically prepared from chickens and rats. Extracts were adjusted to the same protein concentration and injected (0.15 ml/kg) into anesthetized Single Comb White Leghorn roosters. Mean arterial pressure (MAP) and the time for recovery were measured. The most potent extract from chicken hearts was from the left ventricle (-38 ± 1 mm Hg, 149 ± 9 sec to recover). All other extracts (including right ventricle) produced only small (10–20 mm Hg), short-lived (20–30 sec) decreases in MAP. In contrast, only rat atrial extracts evoked long-lasting hypotension (>40 mm Hg, recovery time >200 sec). A 30-min infusion of the most potent chicken extract (left ventricle, CLV) into rats produced a small but significant natriuresis and diuresis compared to the vehicle time control ($P < 0.05$) and the hypotensive response to bolus injection was about one-third that seen in the chicken. The location of potent spasmolytic activity primarily in chicken left ventricle, the different avian renal responses to chicken heart extract and synthetic rat ANP (5), and the weak diuretic, natriuretic, and hypotensive effects of CLV extract in rats all suggest that the chicken heart substance may be different from mammalian ANP.

Recently much attention has been focused on understanding the functions and control mechanisms for synthesis and release of cardiac peptides (atrial natriuretic peptide, ANP). In mammals, most ANP is found in atrial myocytes, but this is not true for all species. Membrane-bound "specific granules" are present in atrial and ventricular cardiocytes of several nonmammalian vertebrates, including chickens (1), and a recent study reported weak ANP immunoreactivity in both atria and ventricles of hens (2).

Little is known about the effects of cardiac peptides in nonmammalian species. In some studies ANP had clear effects on sodium transport. For example, synthetic ANP increased sodium and chloride secretion by the shark rectal gland (3), and inhibited Na–K–Cl cotransport in teleost intestine (4). However, a recent study in chickens (5) showed that infusion of extracts of *whole* chicken heart into chickens had no effect on urine flow rate or absolute or fractional sodium excretion. Instead, it produced marked hypotension and kaliuresis. In contrast, an equivalently hypotensive dose of synthetic rat ANP increased urine flow rate as well as both absolute and fractional sodium excretion.

The purpose of this study was to identify the region(s) of chicken heart containing the hypotensive factor(s) and to compare the vasodepressor activity of extracts of different regions of chicken and rat hearts in chickens. The diuretic, natriuretic, and hypotensive activity of chicken left ventricle extract in rats was also assessed in a preliminary study.

Methods. *Preparation of extracts.* Unanesthetized chickens ($n = 10$) and rats ($n = 8$) were killed by cervical dislocation or decapitation, respectively. Hearts were quickly removed and samples were taken from right and left atria, right and left ventri-

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cles, and intraventricular septum. Similar size samples were also taken from skeletal (breast) muscle and liver. Chicken tissues were weighed, placed in 75 ml 0.1 *N* acetic acid, and frozen. After thawing, the volume was brought to 150 ml and the tissues were homogenized in a Waring blender and left undisturbed for 30 min at room temperature. The homogenates were then boiled for 15 min, cooled, and centrifuged for 30 min at 3600*g*. The lipid layer was removed by aspiration and the remaining supernatant was filtered. The protein concentrations were measured using the Bio-Rad protein assay (Bradford dye-binding technique) and the protein concentration of each was adjusted to 20 μg/ml with 0.1 *N* acetic acid. Extracts were frozen in 1-ml aliquots until used. Rat tissues were treated identically except that the volume of acetic acid was adjusted so that the gram wet weight of tissue per milliliter of acid was the same as for the corresponding chicken tissues.

Experimental animals. Mature Single Comb White Leghorn roosters weighing 1450 ± 50 g (n = 18) were anesthetized by intramuscular injection (3 ml/kg body wt) of quarter-strength allobarbital (Dial, Ciba Pharmaceutical Co.). Supplemental doses were given as required. The common carotid artery was cannulated with PE-50 tubing filled with heparinized avian saline (200 U/ml ammonium heparin) and mean arterial pressure (MAP) was continuously recorded. A wing vein was similarly cannulated for administration of vehicle and extracts.

Experimental protocols: Bird studies. Multiple tissue extracts were tested in each bird, but the order of administration was randomized for each so that the sequence was different for every bird. A constant volume (0.15 ml/kg body wt) of vehicle (0.1 *N* acetic acid) or extract was given except when dose–response curves were done. Parameters measured were maximal change in MAP, time for MAP to return to preinjection value (recovery time), and pre- and postinjection MAP. If MAP did not return to preinjection levels (which was rare), recovery was considered complete when there was no change in MAP for 3 successive min.

Rat studies. Previous studies (5) had shown that chicken heart extracts were not natriuretic and diuretic in chickens, but synthetic rat ANP (arg–atriopeptin III) was. Therefore we tested to see whether chicken heart extracts had any effect on sodium excretion in rats. Only the most potent chicken heart extract (left ventricle) was used because others (6) had already shown that extracts of other chicken tissues or chicken atrial extracts had no natriuretic or diuretic activity in anesthetized rats.

Male Sprague–Dawley rats (250–300 g) were anesthetized by intraperitoneal injection of 150 mg/kg Inactin (Promonta). PE-50 tubing was used to cannulate one jugular vein and one carotid artery. Carotid MAP was recorded continuously. The urethra was ligated and the bladder was cannulated with a 2-cm length of PE-205 tubing. After surgery, an infusion of phosphate-buffered saline (PBS) was started at 0.06 ml/min and a 1-hr recovery period ensued. Rats were then divided into two groups. The vehicle group (n = 5) received vehicle for two successive 30-min periods. The experimental group (n = 7) received vehicle for the first 30 min (control period) and the chicken left ventricle extract (CLV) for the second 30 min. The CLV infusion consisted of 1 ml of lyophilized CLV extract which was redissolved in 1 ml PBS. We verified that the hypotensive activity of the lyophilized extract was unaltered by lyophilization by injecting 0.15 ml/kg of the PBS-dissolved extract into chickens. The infusion rate was always 0.06 ml/min. Parameters measured were urine flow rate, urine osmolality, and sodium excretion rate. Urine flow was determined gravimetrically, osmolality by freezing point depression (Precision Systems Model 5004) and urine sodium by atomic absorption spectophotometry (Varian Technitron Model 1200).

Data analysis. One-way analysis of variance was used to compare the effects of different tissue extracts on both the magnitude and the duration of the fall in MAP. Confidence intervals (95%) based on the pooled variance were constructed for each extract and intervals which did not overlap were considered to be different ($P < 0.05$ or greater). For the rat renal function studies, a paired t test was used to evaluate differences between the first and the second periods within a treatment group. Student's t test was used to evaluate differences between the ve-

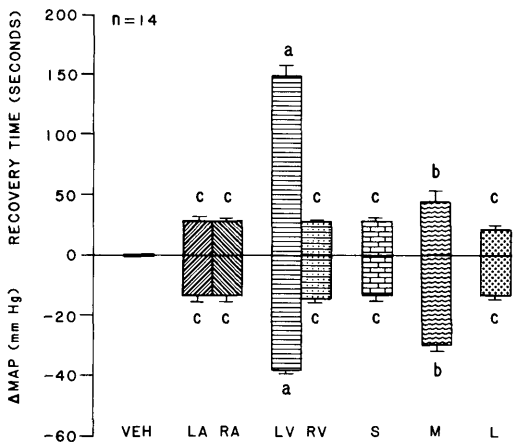

FIG. 1. Effect of extracts of various chicken tissues on mean aterial pressure (MAP) in anesthetized Single Comb White Leghorn roosters. Recovery time is the seconds required for MAP to return to baseline. Mean ± SEM, n = number of birds. VEH, vehicle (0.1 N acetic acid); R, right; L, left; A, atrium; V, ventricle; S, ventricular septum; M, pectoral muscle; L, liver. A constant volume of each extract was given (0.15 ml/kg) and all extracts had the same protein concentration. Bars with entirely different letters are different ($P < 0.05$) from each other. Data were analyzed using one-way analysis of variance (ANOVA). All extracts were different from vehicle alone.

hicle group and the CLV group during any given period.

Results. *Chicken extracts in chickens.* Figure 1 shows the simultaneous decrease in blood pressure and the time required for MAP to return to baseline when extracts of various chicken tissues were injected into chickens. Figure 2 is a representative tracing from one bird. Although all extracts (each having the same protein concentration) had some effect on MAP, only the extract from chicken left ventricle (LV) had a potent, long-acting effect on MAP (−38 ± 1 mm Hg, 149 ± 9 sec to recover; $P < 0.01$ compared to all other extracts). In contrast, right ventricular (RV), as well as atrial (RA, LA), ventricular septal (S), and liver (L) extracts all produced similar small decreases ($P < 0.05$) in MAP which lasted only 25–30 sec. Although breast muscle (M) extract also caused a large decrease in MAP (−30 ± 3 mm Hg), both the magnitude and the recovery time were less than for LV extract ($P < 0.01$ for both) and the shape of the tracing was also quite different (Fig. 2). This hypotensive action of skeletal muscle extract may be due to the acetylcholine which is present in all skeletal muscle, but not to any significant degree in chicken ventricular muscle (7). Further, the marked difference in the hypotensive activity of left and right ventricle extracts supports the contention that the marked hypotensive activity of left ventricle is probably not due to some nonspecific factor.

In some birds, the response to different amounts of the same extract was compared. Figure 3 shows the usual response to increasing amounts of either LV or liver extracts. The hypotensive response to LV was clearly dose dependent, whereas the fall in MAP with liver extracts was little altered by increasing the amount of the injection fourfold.

Rat extracts in chickens. When the experiment was repeated using identically prepared extracts from rat hearts (Figs. 4 and 5), the results were quite different. As expected, extracts from rat atria produced the most significant and long-lasting hypotension in chickens. MAP fell by over 40 mm Hg with either right or left atrial extracts and full recovery from either required slightly more than 4 min. Thus, the effect of mammalian atrial extract was similar in magnitude, but longer in duration than the effect of chicken LV extract. The shape of the tracings was

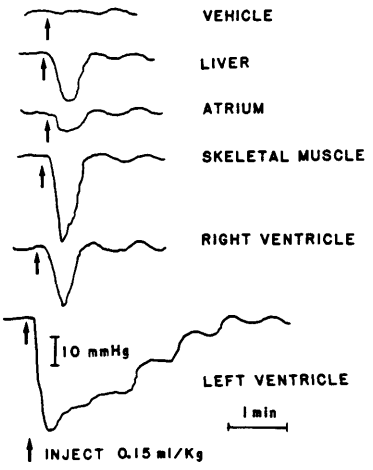

FIG. 2. Comparison of typical chicken MAP response to the same volume of different chicken tissue extracts. All extracts had identical protein concentrations.

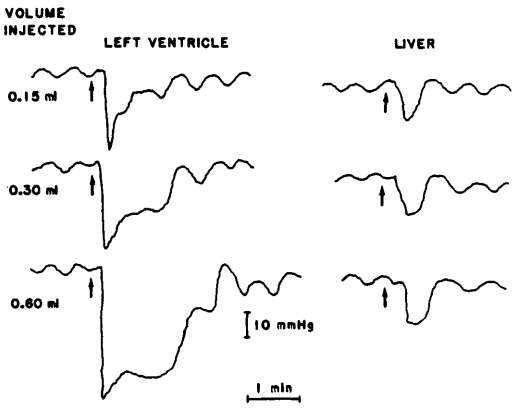

FIG. 3. Representative MAP dose–response relationship for chicken left ventricle (CLV) and liver. Increasing amounts of liver extract had little effect on the size of the hypotensive response, whereas increasing amounts of LV extract caused progressively larger decreases in MAP. Only CLV produced this response. All other extracts (except for rat atria) evoked small, short-term, nonspecific changes in MAP (Figs. 2 and 5).

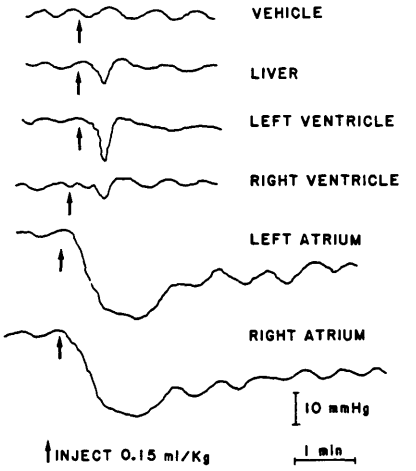

FIG. 5. Comparison of a typical chicken MAP response to the same volume of different rat tissue extracts. All extracts had the same protein concentration and the same amount of tissue wet weight/ml of vehicle as the corresponding chicken tissue extracts.

also somewhat different (compare Figs. 3 and 5). All other rat extracts caused much smaller (10–20 mm Hg), brief (less than 1 min) decreases in MAP. An earlier study with synthetic rat ANP in chickens (5) showed that chickens are profoundly sensitive to the spasmolytic action of ANP. This result and the observation that both rat atria have large numbers of ANP-containing granules (8–10) indicate that the extraction process is adequate. Therefore, the species difference in the location of vasoactive cardiac material is not an artifact of the extraction procedure.

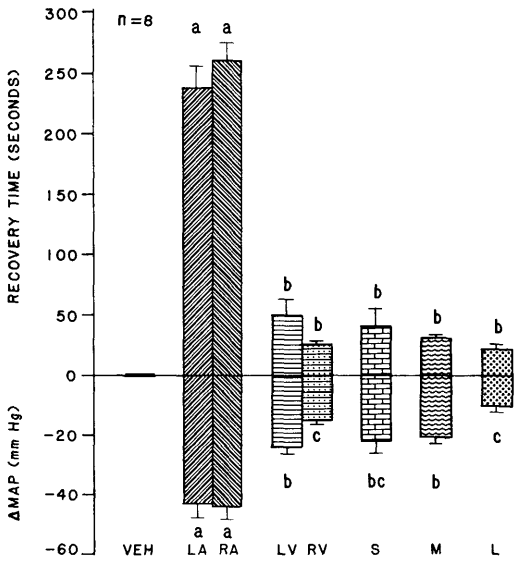

FIG. 4. Effect of extracts of different rat tissues on MAP in anesthetized chickens. See Fig. 2 for details and abbreviations. All extracts were different from vehicle alone.

Effects of chicken left ventricle (CLV) extract in rats. An earlier study showed that extracts of whole chicken heart did not cause natriuresis and diuresis in chickens, but synthetic rat ANP did. Therefore we tested whether the most potent chicken extract had any activity in anesthetized euvolemic rats. Figure 6 shows the absolute changes from a previous 30-min control period in urine flow rate, urine osmolality, and absolute sodium excretion in CLV- and vehicle-infused rats. Control period data are in Table I. Rats infused with vehicle for both periods had no changes in urine flow rate, urine osmolality, or sodium excretion rate, but all three parameters were slightly, but significantly increased by CLV infusion. Bolus injection of CLV into rats at the same dose as was used in chickens (0.15 ml/kg) also caused a much smaller hypotensive effect (-13 ± 2 mm Hg, $n = 3$, compared to -38 ± 1 mm Hg in

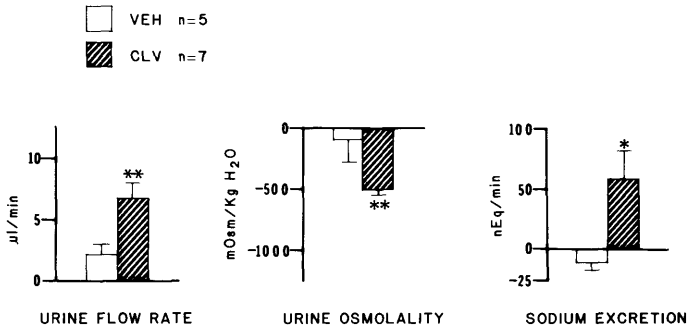

FIG. 6. Effect of a 30-min infusion of chicken left ventricle extract (CLV) or vehicle (VEH) on sodium and water excretion in anesthetized rats. Mean ± SEM. N = number of rats. Data are the change from an immediately prior 30-min control period (vehicle infused). *, ** $P < 0.05$, $P < 0.01$, respectively, test period compared to control period by paired t test.

chickens). Thus, although CLV extract was not as potently hypotensive in rats as it was in chickens (Fig. 1), it did have a small natriuretic and diuretic effect. These effects were not seen when chicken heart extracts were tested in chickens (5).

Discussion. This study demonstrates that in marked contrast to mammals, extracts of chicken left ventricle possess potent hypotensive activity when injected into anesthetized chickens. This response was dose dependent and chickens were more sensitive than rats to the spasmolytic effects of CLV extract. That is, the same dose of CLV on a per kilogram body weight basis lowered rat MAP only 13 ± 2 mm Hg vs 38 ± 1 mm Hg in the chicken. Identically prepared extracts from chicken right ventricle, interventricular septum, or either atrium (all having the same protein concentration) were not different from liver in their ability to lower MAP. This species difference in the location of a vasoactive cardiac factor(s) cannot be due to the manner of extract preparation because identically prepared rat atrial (but not ventricular) extracts were also potently hypotensive in the chicken.

TABLE I. RENAL FUNCTION DURING THE 30-min CONTROL PERIOD

Variable	VEH (n = 5)	CLV (n = 7)
UFR (μl/min)	11.0 ± 1.2	9.4 ± 2.6
UNaV (nEq/min)	39.6 ± 18.3	58.6 ± 15.1
UOsm (mOsm/kg H_2O)	1696 ± 24	1693 ± 151

Note. Vehicle-infused group (VEH) had vehicle infused for two successive 30-min periods. CLV group had vehicle infused during the first 30 min (control period) and chicken left ventricle extract during a second 30-min period. UFR, urine flow rate; UNaV, urinary sodium excretion; UOsm, urine osmolality. There were no differences between the groups during the control period.

An obvious question is whether the CLV hypotensive factor is the same as mammalian ANP. A recent study using a polyclonal antibody to the 8–33 fragment (Arg 101–Tyr 126) of ANP (2) reported weak immunoreactivity in both atria and ventricles of hens, thus suggesting that the granules do contain ANP or a closely related substance. However, weak staining could be due to either a small quantity of immunoreactive material being present and/or a difference in the composition of the granular contents.

On the other hand, physiological data from this and other studies provide rather strong indirect evidence that CLV factor may not be identical to mammalian ANP:

(i) ANP and chicken heart extract have very different renal effects in chickens. Continuous infusion of an acid extract of *whole* chicken heart (0.004 heart equivalents/min × kg) into the renal portal circulation of chickens induced a stable decrease in MAP of 22 ± 7 mm Hg after 10–15 min of infusion, a small unilateral decrease in glomerular filtration rate (GFR), and no change in

sodium or water excretion. In contrast, an equivalently hypotensive infusion of synthetic rat arg–atriopeptin III (−25 ± 8 mm Hg, 27 pmol/min × kg) increased GFR, urine flow rate, and both absolute and fractional sodium excretion. Thus, the inability of chicken heart extract to cause natriuresis and diuresis in chickens was not due to the lack of renal ANP or ANP-like receptors.

(ii) An equivalent dose of CLV extract was only about one-third as hypotensive in rats as it was in chickens, and the effect of CLV on sodium and water excretion in rats was very small compared to the 6- to 90-fold increases which have been reported with rat ANP in mammals (see 9 and 10 for review).

Taken together, these results support the hypothesis that the avian ventricular factor(s) are not mammalian ANP, but analysis of the contents of the chicken cardiac granules is clearly required to clarify this point. The data also strongly suggest the distribution and/or the sensitivities of both renal and vascular ANP or ANP-like receptors are very different in rats and chickens.

The location of a potent chicken hypotensive factor in chicken left ventricle, the profound sensitivity of the chicken to the vasodepressor effects of rat ANP, and the relative insensitivity of chickens to the natriuretic effects of rat ANP (5) all suggest that the function of cardiac factor(s) in chickens may be to regulate blood pressure rather than blood volume. In support of that idea is the report that chicks fed high sodium diets are generally unable to develop an appropriate natriuresis. Instead, they consume large amounts of water and often die. At necropsy ascites, hydropericardium and sometimes anasarca were present in these salt-stressed birds (11, 12). The presence of a potent hypotensive factor in chicken left ventricle may allow chicken blood pressure to be maintained at a more nearly normal level even when there is significant volume expansion due to increased sodium and water retention.

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