PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 181, 277–283 (1986)

Effect of Acute and Chronic Cardiac Denervation on Osmotic Release of Vasotocin in Chickens (42254)

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Abstract. Hypo- and hyperosmotic NaCl were infused intravenously to examine osmotic release of arginine vasotocin (AVT) in anesthetized, acutely cardiac-denervated chickens and in conscious, chronically denervated birds. Mean arterial blood pressure was consistently higher in denervated compared to sham-operated chickens but heart rates were similar in experimental and control groups. Plasma AVT concentrations (pAVT) were significantly higher than controls in acutely, but not chronically, denervated chickens. The slope of the regression line relating pAVT to plasma sodium concentration was higher in denervated birds indicating that removal of cardiac receptor activity increases the osmotic sensitivity of the AVT system. The results suggest that cardiac end-net receptor activity may participate in the regulation of blood pressure and can modulate the release of antidiuretic hormone in the chicken.

The avian heart is unique in that it contains only one type of receptor, the end-net receptor (1). The mammalian heart has both end-net and unencapsulated sensory endings (A and B receptors localized principally in the atrial walls) (2). Functions attributed to mammalian cardiac receptors include regulation of myocardial contractility and vascular pressure and volume by activation of neural and humoral pathways (3, 4, 5). There is also evidence that mammalian left atrial B-type receptors may influence the release of antidiuretic hormone (ADH) (6, 7). Thus, stretching the left atrium inhibits neurosecretory neurones in the paraventricular and supraoptic nuclei (8) and plasma vasopressin levels (pAVP) are inversely related to left atrial pressure and B-type receptor activity (7). Less attention has been paid to the role end-net sensory fibers might play in regulating visceral function.

Although the function of avian end-net receptors is unknown, stimulation of the vagal rami carrying those sensory fibers does cause changes in ventilation, heart rate, and blood pressure (1). The present study was undertaken to determine whether the activity of avian cardiac end-net receptors influences the release of arginine vasotocin (AVT), the avian ADH (9). The results observed following denervation of the heart suggest that the end-net receptor system may tonically inhibit the release of AVT in chickens.

Materials and Methods. Single Comb White Leghorn pullets or cockerels, weighing 0.48 to 0.91 kg, were used in these studies. The birds were individually caged in an environmentally controlled room with an average temperature of 22°C and a 12-hr light cycle and were maintained on water and food *ad libitum*. The animals were adapted to their cages for at least a week before being used.

Chronic cardiac denervation. Three-week-old male chickens were anesthetized with sodium pentobarbital (35 mg/kg ip). They were secured on their backs on a heating pad to maintain body temperature at 41°C. A midline incision was made along the left side of the keel and the skin and muscle layers were deflected laterally to expose the bone. The sternum was cut preserving the air sacs not attached to the bone. The thoracic cage was retracted laterally and the birds provided with 100% oxygen. The heart was denervated by cutting the right middle cardiac nerve and the right vagus nerve caudal to the pulmonoesophageal and recurrent nerves. The left middle and posterior cardiac nerves were sectioned leaving the vagal innervation to the lungs and abdominal viscera intact. The cut edges of the sternum were approximated and secured with stainless steel surgical wire and

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0037-9727/86 $1.50

the incisions were closed with sutures. An antibiotic (Combiotic, Pfizer, 0.1 mg/100 g body wt, im) was administered immediately following the surgical procedure and once daily for the subsequent 3 days. Sham-operated controls were treated identically without cutting the nerves.

Experiments were performed on eight denervated and six sham-operated chickens [0.58 ± 0.014 (SE) kg body wt] approximately a month following denervation of the heart. Chickens, allowed water *ad libitum* and fasted overnight, were hooded and secured on their backs. Under local anesthesia (Procaine HCl) cannulas were inserted in both wing arteries to monitor arterial blood pressure and for withdrawing blood samples. A wing vein was cannulated for infusion of saline.

Acute cardiac denervation. Twelve pullets with an average body weight of 0.73 ± 0.33 kg were used to observe the effects of acute denervation of the heart. Birds, fasted overnight but allowed water *ad libitum,* were anesthetized with sodium pentobarbital (30 mg/kg). They were tied on their backs on a heating pad and the thoracic and abdominal cavities were opened. The air sacs were broken and the birds were ventilated by passing warmed (41°C), humidified gas (5% CO_2, 95% O_2) unidirectionally through the lungs at a rate of approximately 1 liter/min. Respiratory movements were suppressed by injection of 2 mg gallamine triethiodide (Flaxedil, American Cyanamid Co.) and supplemental anesthetic (about 5-mg dose) was provided every half hour. The heart was denervated by cutting the nerves as described for the chronic denervation studies (above). Cannulas were placed in a wing vein (for infusion) and both wing arteries (for blood sampling and pressure measurements).

Experimental protocol. Similar protocols (saline infusion, physiological measurements, and blood sampling) were used to study conscious, chronically denervated and anesthetized, acutely denervated chickens. The birds were allowed to equilibrate for 20–30 min following completion of the surgical procedures before measurements were begun. After obtaining a blood sample (2 ml) in a chilled, heparinized syringe, 0.1 *M* NaCl, followed by 1.0 *M* NaCl, was infused for 30 min each at a rate of 0.5 ml/kg/min. Five additional blood samples were obtained every 10 min beginning 20 min after starting the saline infusion. Measurements of blood pressure and body temperature were recorded immediately before obtaining each blood sample.

Mean (MABP) and pulsatile arterial pressures were measured with a Statham strain gauge (P23AA) connected to a pen recorder (Beckman Model RB Dynograph). Heart rate (HR) was calculated from the arterial pressure tracing. Body temperature was monitored by a thermistor probe inserted into the colon and attached to a telethermometer.

Analysis of samples. Immediately after obtaining each sample the blood was centrifuged for 5 min at about 2000*g*. Half-milliliter aliquots of plasma were stored at −20°C for measurements of AVT. The remaining plasma was used for determination of sodium by specific ion electrode (Technicon Instruments).

Radioimmunoassay of AVT was performed using the assay method described earlier (10). Free and bound labeled hormones were separated by the polyethyleneglycol (PEG) method (11). Fifty microliters 1.5% bovine γ globulin (Cohn Fraction II, Sigma) and 500 μl 25% PEG were added to the tubes. The tubes were vortexed and centrifuged at 2000*g* for 60 min at 4°C. The supernatant was aspirated and the precipitate was counted on a Packard autogamma counter (Model 5235). All AVT values in the present study were obtained in a single assay with an intraassay coefficient of variation of 6.2% (N = 8). Recovery of standard AVT from aliquots of pooled plasma averaged 85 ± 9.8% (N = 4). Plasma AVT values were not corrected for recovery. For statistical analysis of the AVT data, the minimum detectable dose (1 μU/ml) was used where ever a value was less than the limit of detection of the assay.

Statistical analysis. A one- or two-factor analysis of variance for repeated measures was performed on the data using the statistical analysis system (SAS) of Barr *et al.* (12). Differences within and between groups were isolated by the paired and unpaired t tests. Correlation coefficients were calculated using the CORR procedure described in SAS. The data are expressed as means ± SE.

Results. *Chronic cardiac denervation.* Prior to saline infusion MABP in unanesthetized, chronic, cardiac-denervated chickens (182

± 6.9 mm Hg) was higher than in sham-operated controls (153 ± 14.2 mm Hg). Although the differences were not statistically significant, higher blood pressures were observed in the denervated group throughout the experiment (Table I). Hyperosmotic saline (1.0 *M* NaCl) infusion in denervated chickens resulted in a 10 to 15% decrease in MABP compared to the preinfusion mean ($P < 0.01$ or 0.05). Smaller reductions in pressure (1 to 10%) were also observed in sham-operated controls during 1.0 *M* NaCl infusion ($P > 0.05$). Initial HR in denervated and control groups were similar and the changes that occurred during saline infusion were not statistically significant (Table I). Compared to rates observed at the end of 0.1 *M* NaCl infusion, the hypotension resulting from infusion of hyperosmotic NaCl was associated with a 9% increase in HR in controls and a slight (3%) decrease in denervated birds.

Figure 1B summarizes the effects of saline infusion on plasma sodium (pNa) and AVT levels (pAVT) in the chronically cardiac-denervated chickens. Preinfusion means for plasma sodium in denervated and control groups were 141 ± 1.1 and 143 ± 1.9 meq/liter, respectively ($P > 0.05$) and increased by 20 meq/liter in both groups at the end of 1.0 *M* NaCl infusion (*P* values < 0.01). In denervated birds infusion of 0.1 *M* NaCl caused

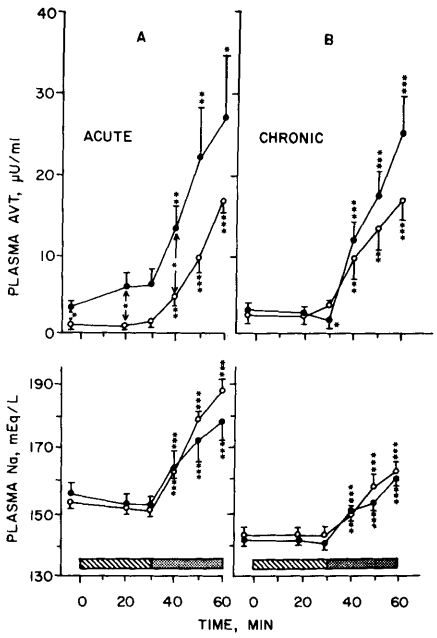

FIG. 1. Effect of infusing hypo- and hyperosmotic saline on plasma concentrations of AVT and Na in cardiac-denervated (solid circles) and sham-operated chickens (open circles). (A) Acute denervation; (B) chronic denervation. Infusion of 0.1 and 1.0 *M* NaCl is indicated by the hatched and stippled bars, respectively. Means ± SE. Asterisks above and below symbols indicate significant differences from preinfusion means. Asterisks between symbols indicate significant differences between denervated and control groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

TABLE I. MABP (mm Hg) AND HR (BEATS/MIN) IN ACUTE AND CHRONIC, CARDIAC-DENERVATED CHICKENS AND THEIR SHAM-OPERATED CONTROLS DURING INTRAVENOUS SALINE INFUSION

Experimental group	Parameter	Time (min) 0	20	30	40	50	60
Chronic preparation							
Denervated	MABP	182 ± 6.9	184 ± 8.2	181 ± 6.7	164 ± 10.7[a]	158 ± 11.1[b]	154 ± 10.6[b]
	HR	343 ± 16	333 ± 15	326 ± 14	313 ± 14	313 ± 11	318 ± 9
Control	MABP	153 ± 14.2	161 ± 10.0	159 ± 12.2	152 ± 8.0	144 ± 6.5	138 ± 5.9
	HR	347 ± 37	304 ± 33	312 ± 27	340 ± 29	339 ± 20	341 ± 10
Acute preparation							
Denervated	MABP	112 ± 11.9	94 ± 8.6	106 ± 5.6	104 ± 8.3	102 ± 7.9	98 ± 9.0
	HR	348 ± 11	378 ± 10	370 ± 8	368 ± 11	365 ± 12	356 ± 8
Control	MABP	91 ± 9.7	84 ± 11.0	83 ± 10.2	103 ± 10.3[b]	108 ± 12.1[a]	109 ± 7.8[a]
	HR	358 ± 16	362 ± 18	362 ± 18	358 ± 19	352 ± 21	352 ± 22

Note. Column under 0 min indicates values immediately before infusion. NaCl (0.1 and 1.0 *M*) was infused from 0 to 30 and 30 to 60 min, respectively. Means ± SE. $N = 6$ per group except for acute, sham-operated controls ($N = 5$).
[a] $P < 0.05$; [b] $P < 0.01$ (compared to 0-min mean).

pAVT to decrease from 3.1 ± 0.88 to 1.8 ± 0.40 μU/ml. The reduction in pAVT was moderate but statistically significant ($P < 0.05$). The slight increase in pAVT in sham-operated controls over the same time period (2.6 ± 1.1 to 3.4 ± 1.8 μU/ml) was not significant. Compared with preinfusion levels, pAVT at the end of 1.0 *M* NaCl infusion were elevated by 21.9 ($P < 0.01$) and 13.9 μU/ml ($P < 0.01$) in experimental and control groups, respectively.

The correlation coefficients relating pAVT to pNa in denervated and sham-operated controls (Fig. 2B) were 0.81 ($N = 48$) and 0.64 ($N = 36$), respectively (P values < 0.001). The regression line describing the relationship in the experimental birds, pAVT = 1.05 (pNa—138), was steeper than in the control group, pAVT = 0.47 (pNa—133), and the difference in slopes was statistically significant ($P < 0.001$).

Acute cardiac denervation. MABP values were lower in anesthetized, open-chest chickens compared to conscious chronically operated birds, averaging 91 ± 9.7 mm Hg (control) and 112 ± 11.9 mm Hg (denervated) (Table I). In sham-operated controls, blood pressure increased 13 to 20% during 1.0 *M* NaCl infusion (P values < 0.01 or 0.05) while pressures in denervated birds declined moderately. Changes in HR were not statistically significant.

Plasma Na concentration increased from 156 ± 4.0 to 178 ± 5.7 meq/liter ($P < 0.01$) in acutely denervated birds and from 153 ± 1.3 to 188 ± 2.9 meq/liter ($P < 0.01$) in sham-operated controls (Fig. 1A). Plasma AVT increased to 26.8 (denervated) and 17.1 μU/ml (controls) from preinfusion means of 3.5 and 1.4 μU/ml, respectively. Statistically significant differences in pAVT levels were also observed between denervated and control groups at 0, 20, and 40 min. The correlation coefficients of pAVT with pNa were 0.56 ($N = 36$) in acutely denervated chickens and 0.87 ($N = 36$) in sham-operated controls (P values < 0.001). The regression lines relating pAVT to pNa were pAVT = 0.55 (pNa—139) and pAVT = 0.35 (pNa—146) for denervated and control groups, respectively (Fig. 2A). The difference in slopes between the two groups was not statistically significant.

Discussion. In mammals, changes in blood volume and arterial pressure can affect the hy-

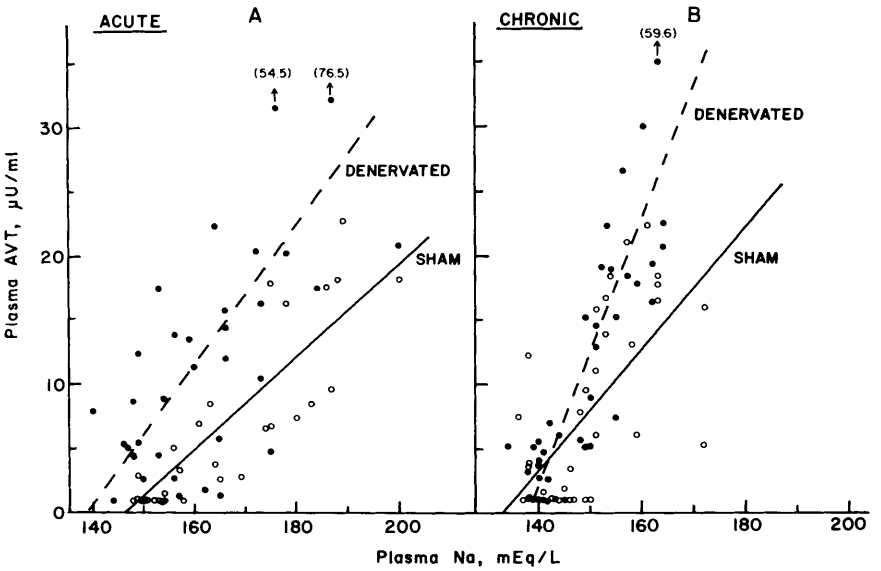

FIG. 2. Relationship between plasma AVT and Na concentrations in cardiac-denervated (solid circles) and sham-operated control chickens (open circles). (A) Acute denervation ($N = 36$) and sham-operated controls ($N = 36$); (B) chronically denervated birds ($N = 48$) and controls ($N = 36$). Slopes of the regression lines of experimental and control birds were significantly different ($P < 0.01$) for the chronic preparations. Differences in x-intercepts between experimental and control groups were not statistically significant.

pothalamo-neurohypophyseal system by alterations in afferent activity of atrial and arterial mechanoreceptors (6, 7, 8). The firing frequencies of arterial baroreceptors and left atrial B-type receptors decrease in response to hypotension and reductions in atrial filling, respectively (13). Decreases in high- and low-pressure mechanoreceptor activities are associated with increases in plasma vasopressin levels (7). In addition, stimulation of ventricular receptors with cryptenamine in dogs (14) or electrical stimulation of the nonmedullated afferents from these receptors in cats (15) also inhibits the release of ADH.

Sensory information from cardiovascular mechanoreceptors project to the nucleus of the solitary tract (NTS) in the medulla (16, 17). The cardiovascular sensory neurons in the NTS regulate ADH secretion either via projections to the Al cell group in the ventral medulla (18) or by a direct pathway to the paraventricular and supraoptic nuclei (16, 17). It is the Al region which appears to provide the principal pathway for visceral afferent control of ADH release (18).

Functional arterial baroreceptors have not been identified in the head and neck of chickens, including the homolog of the carotid sinus (19). While the chicken and other nonmammalian vertebrates lack complex unencapsulated receptors (cardiac A and B receptors) (2) ventricular end-net receptors with activity patterns similar to mammals have been described in this avian species (20). The activity of the fowl ventricular receptors increases with pressure and may contribute to reflex regulation of blood pressure (21).

Knowledge of cardiovascular reflexes and pathways are not well understood in the chicken. Thus, methods for testing completeness of cardiac denervation, such as those used in mammals (22, 23, 24), are not available for the fowl. Cardiac denervation is simpler to perform in chickens, however, since receptor afferents from the heart are carried by discrete vagal rami that are easily isolated (1, 20).

The middle cardiac nerve in chickens contains mainly afferent fibers from the ventricular walls. Since section of this nerve bilaterally results in abrupt increases in arterial pressure (1) we have used pressure changes as our criterion to determine completeness of denervation. Although not statistically significant, MABP of both chronic and acutely denervated chickens were about 20% higher than in sham-operated controls (Table I). The pressure increases following cardiac denervation suggest that mechanoreceptors in the heart participate in the regulation of arterial pressure and that mechanisms for compensatory adjustment of pressure are absent following denervation in the fowl. The absence of such compensatory adjustments is also consistent with the apparent lack of functional arterial baroreceptors in this species (19). In contrast, arterial pressure changes are not observed following cardiac denervation in dogs (23, 24) or in a nonhuman primate (25).

One way of assessing cardiovascular mechanoreceptor inputs to the ADH system in mammals is to observe the effects of changes in blood volume or arterial pressure on the osmotic sensitivity (pADH-pOsm relationship) of the osmoregulatory system (26, 27, 28). For example, the effect of blood volume contraction on the neurohypophysis in rat (26), man (27), and dog (28) is manifested by a reduction in osmotic threshold and/or an increase in osmotic sensitivity of the system.

The increase in slope of the line relating pAVT and pNa in acute or chronic cardiac-denervated chickens (Fig. 2) suggests that the activity of cardiac mechanoreceptors tonically inhibits the release of AVT. Inhibition of AVT release by cardiac end-net receptor activity is also consistent with the higher pAVT levels that are observed in acutely denervated birds compared to sham-operated controls (Fig. 1A). Differences in pAVT levels between chronically cardiac-denervated birds and controls, however, were not seen (Fig. 1B). The reason for this is not clear. The experiments using chronically denervated chickens were performed a month following surgery. One possibility is that unidentified, compensatory mechanism in the chronic preparation have had time to correct the differences in pAVT that may initially be caused by denervation. Similar basal plasma ADH levels have also been observed in conscious, cardiac-denervated, and sham-operated dogs (23, 24).

In mammals, cardiovascular regulation of pADH is determined by the relative influence of arterial and cardiac baroreceptor activity (29). In dogs, the changes in osmotic sensitivity of the ADH system resulting from hypo- and

hypervolemia are abolished following cardiac denervation (23, 24). This suggests that cardiac receptors can play an important role in volume regulation of ADH release under certain circumstances. In water-deprived dogs, however, the reduction in volume is less important than the increase in osmolality in stimulating AVP release (30).

What is known of cardiovascular reflexes in the fowl and the results of the present study suggest that cardiac end-net receptors can mediate volume-induced alterations in AVT release. Whether cardiac baroreflexes influence AVT secretion under physiological conditions remains to be established. Indeed, data from our laboratory (31) and others (32, 33) indicate that hemorrhage (20 to 50% of the blood volume) is not a stimulus for AVT release in chickens. Additional experiments are needed to identify the conditions that determine the level of cardiac baroreflex input to the ADH system in the fowl.

Supported by American Heart Association, Arkansas Affiliate, and in part by NSF PCM-8316015. We are indebted to Dr. H. Artman and Dr. D. A. Fisher for a generous supply of AVT antiserum (R70), and to Dr. W. H. Sawyer for assaying our AVT standard.

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Received January 28, 1985. P.S.E.B.M. 1986. Vol. 181.
Accepted October 15, 1985.