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Catecholamine-Mediated Diuresis in Turkeys[1] (41114)

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Abstract. A catecholamine-mediated mechanism that may operate in water homeostasis of turkeys is described. Administration of epinephrine to turkeys in dosages that had few, or no, pressor or chronotropic effects (<4.0 μg·kg body wt^{-1}) stimulated urine flow. Increases in GFR and an unchanged urine to plasma inulin ratio were temporally related to the diuresis. These data suggest that epinephrine increases the number of functioning reptilian-type nephrons. Since this population of nephrons has little capacity for concentrating the glomerular filtrate the number of nephrons that are functional at a given time is important for water balance. Epinephrine, norepinephrine, and dopamine produced diuresis, but epinephrine was most potent.

Pharmacologic doses of the β-adrenergic agonist, isoproterenol hydrochloride (isoproterenol), have a diuretic effect in birds in contrast to an antidiuretic effect in mammals (1–6). The mode of action and the physiologic significance of this effect in birds are the subject of this report.

Regulation of the renal glomerular filtration rate (GFR) is more important for water homeostasis in birds than in mammals in whom homeostasis is primarily maintained by water reabsorption from the renal tubules (7–10). Thus, a possibility exists that the diuresis observed in birds following acute administration of isoproterenol results from an increased renal tubular water load due to an elevated GFR. The avian kidney contains two types of nephrons, a mammalian (MT) and a reptilian (RT) type (7, 8). The MT concentrates urine by a mechanism involving a counter-current system, as in mammals (11). The RT lacks a counter-current organization and has little capacity for water conservation (8). Thus, in birds, water homeostasis is achieved, in part, by regulating the number of functioning RT nephrons (8, 9, 12) which is reflected by changes in the GFR (8, 9). In this report, evidence is presented which indicates that catecholamines are important in controlling the GFR and, hence, the number of functioning RT nephrons.

Materials and Methods. *General.* Seven male, broad-breasted, white turkeys were studied repeatedly over a period of 6 months. During this time, the turkeys grew from 6.0 ± 1.6 to 15.6 ± 2.0 kg. Although this represents a large variation in body weight, each turkey was used as its own control in all studies. Methods of restraint and urine collection were described earlier (13). When renal clearances were to be measured, an 18-gauge, 2-in. canine indwelling catheter[2] was inserted in a vein of each wing. Injections and infusions were made in one catheter; blood samples were collected from the other.

GFR was measured by inulin clearance (C_{in}) as follows. A 700 mg/kg priming dose of inulin (100 mg/ml) was injected iv 45 min before the experiment began. Inulin (35 mg/ml) in isotonic saline was then infused (7 mg/min) continuously until the experiment ended. Ten milliliters of blood was collected midway in each 15-min clearance period. Inulin concentrations in plasma and urine were measured for each clearance period by the method of White and Samson (15). Clearances (C) were calculated by the following formula: C = urine volume × urine inulin concentration ÷ plasma inulin concentration.

Experiment 1. The effects of epinephrine on GFR and the temporal relation of these

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[2] Sherwood Medical, St. Louis, Mo.

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effects to urine production were determined after the birds were restrained, catheters inserted, and infusions started as described above. Forty-five minutes was allowed before the first of five successive 15-min C_{in} measurements were made. After the second clearance period (30 min), either epinephrine (4.0 or 40 μg/kg body wt) or saline in a 1.0-ml volume was injected into the infusion tubing and the effects were measured in the remaining three postinjection C periods. On another day, within 2 weeks, the treatments were reversed. Hence, each bird served as its own control.

In three experiments in which epinephrine was injected, heart rate by electrocardiogram (EKG), and urine flow rates were measured. On another occasion, 0.4 and 4.0 μg epinephrine/kg body wt were injected into six birds anesthetized with sodium pentobarbital, and the effects of the catecholamine on systemic arterial pressure and heart rate were measured. A Narco-Bio-Systems polygraph and either intraischiatic or intracarotid arterial catheters were used for this purpose.

Experiment 2. In a series of 17 different experiments, the dose–response relationship between the dose of catecholamine administered and urine flow was determined. (See Table II for dosages and the number of experiments in which each drug was used.) Epinephrine,[3] isoproterenol,[4] dopamine,[5] and norepinephrine[6] were studied. Turkeys were infused throughout the experiment with isotonic saline at a rate adjusted to replace the volume of urine excreted. After an initial equilibrium period to allow urine flow to stabilize (30–45 min), one of the above catecholamines was injected iv in 1 ml of saline. Afterwards, the urine flow rate was measured at 5-min intervals for 15 min or until the urine flow returned to preinjection control levels, whichever was longest. At this time another catecholamine dosage was administered and the process of measuring the urine flow rate was repeated. Due to the variation in time necessary for urine flow rates of individual birds to become maximal, both at a given catecholamine dosage and for different dosages, urine flow rates were derived from the 5-min interval in which urine flow was maximally stimulated.

Results. *Experiment 1.* Administration of 4.0 μg epinephrine/kg body wt was associated with increases in urine flow and concomitant increases in C_{in} and osmolar clearances (C_{osm}) ($P < 0.05$) (Table I). Free water clearances (C_{H_2O}) were negative and became more negative with time in both epinephrine- and saline-injected birds. There were no significant changes in the ratio between urine and plasma inulin concentrations (U/P_{in}). Increases in C_{in} did not occur when 40 μg epinephrine/kg body wt was injected, although urine flow increased in four of five birds (from 37 ± 10 to 146 ± 46 μl/min/kg body wt). In other experiments, this dosage produced marked cardiac arrhythmias.

Doses of epinephrine that induced diuresis and stimulated GFR (Tables I and II) had no consistent effects on systemic arterial pressure, a variable which could mediate the renal responses (Fig. 1). Injections of 0.4 μg of epinephrine/kg body wt did not alter the heart rate or blood pressure significantly. Neither were there discernable changes in the EKG pattern. Within 1 min after injection of 4.0 μg of epinephrine/kg body wt arterial blood pressure increased in some but not all birds. While no significant differences existed in blood pressure, heart rate decreased ($P < 0.05$) from an average of 200 to 178 beats/min. Thereafter, the average heart rate increased progressively to control levels, but no significant differences were observed after the first minute. Doses of 40 μg/kg body wt resulted in a characteristic ruffling of feathers, hyperexcitability, and dramatic cardiac arrhythmias which lasted for 10 min or more. The effects of norepinephrine (4.0 and 40 μg/kg body wt) on the heart rate resembled those of epinephrine at the same doses.

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[3] Epinephrine HCl injection (1:1000), Interstate Drug Exchange, Plainview, Long Island N.Y.

[4] Isoproterenol hydrochloride, Aldrich Chemical Company Milwaukee, Wisc.

[5] 3-Hydroxytyramine HCl, Sigma Chemical Company, St. Louis, Mo.

[6] Levophed bitartrate, Winthrop Labs, N.Y.

TABLE I. RENAL FUNCTION IN TURKEYS INJECTED WITH EPINEPHRINE OR SALINE[a]

Injectate	Preinjection (min)		Postinjection (min)		
	30	45	15	30	45
Inulin clearance (μl/min/kg)					
Saline (9)[b]	520 ± 80	570 ± 80	590 ± 80	570 ± 70	560 ± 60
Epinephrine (7) (4 μg/kg)	700 ± 50	690 ± 80	920 ± 80[c,d]	820 ± 50[d]	740 ± 40[d]
Urine flow (μl/min/kg)					
Saline (9)	47 ± 17	40 ± 12	32 ± 8	31 ± 6	24 ± 4
Epinephrine (7) (4 μg/kg)	37 ± 7	33 ± 6	55 ± 9[e,f]	50 ± 12	38 ± 8
Osmolar clearance (μl/min/kg)					
Saline (7)	46 ± 15	52 ± 18	40 ± 8	48 ± 10	43 ± 8
Epinephrine (5) (4 μg/kg)	52 ± 7	48 ± 5	70 ± 9[d]	85 ± 16	66 ± 5[d]
Urine [inulin]:plasma [inulin]					
Saline (10)	25 ± 6	28 ± 7	29 ± 5	31 ± 7	30 ± 6
Epinephrine (8) (4 μg/kg)	27 ± 6	27 ± 6	27 ± 8	34 ± 10	31 ± 7

[a] Mean ± SE.
[b] Numbers in parentheses = n.
[c] Significantly different compared to 45 min preinjection control ($P < .025$ by one tailed t test).
[d] Significantly different compared to saline injected controls ($P < .025$ by one tailed t test).
[e] Significantly different compared to 45 min preinjection control ($P < .05$ by one tailed t test).
[f] Significantly different compared to saline injected controls ($P < .05$ by one tailed t test).

Experiment 2. Diuresis occurred when turkeys were injected iv with all of the catecholamines studied (Table II). Birds were most sensitive to epinephrine and isoproterenol, and least sensitive to dopamine. Norepinephrine was effective at 4.0 and 40 μg/kg body wt. In some birds, epinephrine and isoproterenol at doses of 0.004 and 0.04 μg/kg body wt induced a small diuresis almost immediately after

TABLE II. URINE FLOW IN TURKEYS INJECTED iv WITH CATECHOLAMINES

Dose (μg/kg/body wt)	Isoproterenol	Epinephrine	Norepinephrine	Dopamine
Saline	0/3[a]	0/3	0/2	ns
0.004	2/4 (29 ± 17)[b]	1/1 (12)	ns	ns
0.04	2/4 (32 ± 7)	1/1 (81)	0/2	ns
0.4	7/8 (50 ± 10)	5/5 (48 ± 11)	0/2	0/1
4.0	6/6 (69 ± 15)	6/7 (54 ± 7)	1/2 (37)	0/1
40.0	6/6 (162 ± 33)	4/5 (146 ± 46)	2/2 (78 ± 22)	0/1
400.0	1/1 (850)	0/1	ns	3/3 (32 ± 3)
800.0	ns[c]	ns	ns	2/2 (54 ± 9)
1200.0	ns	ns	ns	2/2 (82 ± 38)

Note. Repeat dosages in individual birds often were quantitatively more effective than the initial dose. Epinephrine and isoproterenol doses of 4.0 μg/kg or less had effects that usually lasted less than 10 min. Larger doses had effects that lasted approximately 30 min and produced marked EKG changes. Comparable dopamine dosages produced an effect which lasted a much shorter period, and the rate of urine flow was relatively small. Norepinephrine was tested in different birds than the other catecholamines.
[a] Number of responding birds/number of birds injected.
[b] Mean increase in urine flow (μl/min/kg) ± SEM during the 5-min interval in which urine flow rate was highest.
[c] Not studied.

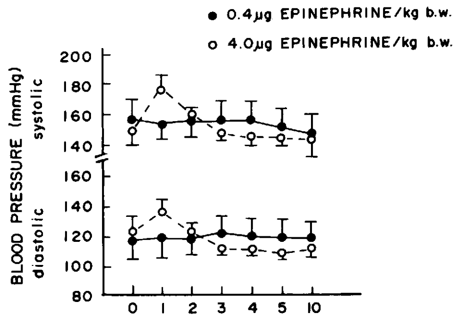

FIG. 1. Carotid artery pressure in turkeys injected intravenously with epinephrine (mean ± SEM, $n = 6$). In the 4.0 μg/kg dosage group n varied in some time intervals because recordings were disrupted by a turkey struggling. However, no fewer than four records for each interval were obtained. The paired t test revealed no significant differences.

the injection. This diuresis lasted less than 10 min. A more consistent, and quantitatively greater, increase in urine flow (three to six times preinjection controls) occurred after injections of 0.4 and 4.0 μg/kg body wt. These responses occasionally lasted 15 min or more but usually for less than 10 min. Marked increases in urine flow lasting 30 min or longer occurred at doses of 40 μg/kg body wt. Effects of epinephrine on urine flow lasted much longer than effects on the heart rate and systemic arterial pressure (Fig. 1).

Discussion. Objectives of these experiments were to determine whether (a) the endogenously produced catecholamines, i.e., epinephrine, norepinephrine, and dopamine, induce a diuresis in turkeys as has been reported for isoproterenol (1, 2); and (b) an increase in the GFR is associated with catecholamine-induced diuresis.

Epinephrine and norepinephrine had diuretic effects similar to those of isoproterenol. Dopamine was effective only in large, unphysiologic doses (400 μg/kg body wt). Diuresis occurred following administration of either epinephrine or norepinephrine at doses which produced few, if any, cardiovascular effects. Hence, the effects of catecholamines on urine flow is potentially important physiologically. Thus, in birds, catecholamines appear to induce a renal response opposite to that produced by arginine vasotocin, the avian antidiuretic hormone (ADH). The effects of catecholamines on the avian kidney is similar to that induced by a water load, i.e., diuresis and increased GFR (12, 14). This suggests, but does not prove, an interplay between ADH and endogenous catecholamines in the body fluid homeostasis of these animals.

Cathecholamines probably induce diuresis, at least partly, by increasing the numbers of functional RT nephrons. Others have reported that changes in the overall GFR in birds are associated with parallel changes in the numbers of functioning RT nephrons which have a low-concentrating capacity (7). Hence, the increased GFR after relatively low doses of epinephrine, in conjunction with the previously reported dilatation of renal tubules (1), suggests an increase in the number of poorly concentrating RT nephrons. Other data also support this hypothesis. The U/P_{in} did not change after epinephrine (4.0 or 40 μg/kg body wt) despite the increased urine flow. If the diuresis resulted from decreased water reabsorption by individual nephrons, the ratio would decrease since U_{in} would be distributed in a larger volume of unreabsorbed filtrate. The increased C_{osm}, but unchanged urine and plasma osmolality, is also consistent with the effect due to increased numbers of functional nephrons.

When pharmacologic doses of catecholamines are administered, additional factors may become operative. Large doses of epinephrine (40 μg/kg body wt) were strongly diuretic ($P < 0.01$), but had no measurable effect on GFR. The accompanying myocardial arrhythmia suggests alterations in the cardiovascular system which could preclude increases in GFR. Large doses of isoproterenol (400 μg/kg body wt) injected subcutaneously also produced a diuresis which was not temporally related to GFR, although increases in C_{in} did occur (Palmore, unpublished experiments). Diuresis, in the absence of effects on GFR, could be the consequence of opposing effects of the dose of catecholamines on MT and RT nephrons. For the reasons stated above, the number of low-concentrating RT nephrons appeared to be increased. However, epinephrine is known to reduce GFR in mammals (16) and may have a similar effect on MT nephrons in birds. Catecholamines are also reported to divert blood from the avian portal circulation (17), but the extent to which this affects urine flow and GFR is unknown.

In summary, physiologic doses of the endogenously produced catecholamines, epinephrine and norepinephrine, increased urine flow rate and GFR in turkeys. The fact that relatively low doses of catecholamines affect both urine output and GFR suggests that they may play an important role in the maintenance of water balance in turkeys.

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