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Isoproterenol-Induced Diuresis in Turkeys[1] (39727)

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We reported earlier that sc administration of *d,l*-isoproterenol sulfate dihydrate and *d,l*-isoproterenol hydrochloride (isoproterenol; Aldrich Chemical Co., Milwaukee, Wis.) to turkeys stimulated the flow of urine (1). The observation was surprising because isoproterenol, administered to mammals, has a strong antidiuretic effect (2–7). The mode of action in mammals may be directly on renal tubules to stimulate cAMP and thus mimic the effect of antidiuretic hormone (ADH) (6, 8, 9), or by way of stimulating ADH secretion (10, 11).

Because of the striking difference in the renal responses of mammals and the turkey to isoproterenol, the effects of this β-adrenergic agonist on renal hemodynamics, fluid and electrolyte balance, and systemic circulation were studied. The results indicate that the isoproterenol-induced diuresis is associated with an increased glomerular filtration rate (GFR) and increases in free-water clearance (C_{H_2O}).

Methods. Six, 5-month-old, broad-breasted white turkey hens with a mean weight of 7.6 ± 0.2 kg were studied. They were fed a commercial poultry ration and watered *ad libitum* in a communal pen for 6 weeks before the experiment began. Each bird was placed in a stainless steel cage for 16 hr without food or water before measuring renal clearances.

Experimental protocol. At the outset of experiments on individual birds, 480 mg of *p*-amino hippuric acid (PAH) and 2.7 g of creatinine (Cr) in 30 ml of distilled H_2O were injected sc into the neck. The bird was then restrained on a dog board and the head was covered with a towel. Birds usually lay quietly during the 2.5 hr procedure without use of anesthesia. In the 30 min before clearance measurements were started, the cloaca was emptied by a combination of flushing with saline, wiping with saline-moistened gauze squares, and aspiration (see below). Cloacal emptiness was determined visually with the use of a canine vaginal speculum. After gently stretching the cloacal orifice with the finger, urine emission from the ureters was seen and was collected at 4- or 5-min intervals in a filtering flask trap by aspiration with a vacuum pump. The aspiration tube (NCG oxygen catheter, National Cylinder Gas, Chicago, Ill.), attached to rubber tubing, contained perforations which excluded bits of feces. Between the sequential clearance measurements, the system was rinsed with 10 ml of isotonic saline. Later, urine volumes and Na excretion were corrected for this amount.

Thirty minutes after the Cr-PAH injection, the following clearance measurements were made: creatinine (C_{Cr}), PAH (C_{PAH}), sodium (C_{Na}), osmolal (C_{Osm}), and free water (C_{H_2O}). Ten milliliters of venous blood were collected by venipuncture from the wing vein midway in each 30-min interval. The concentration of the respective solutes in this sample was used to calculate clearances.

Creatinine and PAH were assayed by routine methods (12), Na and K were assayed by flame photometry, and osmolality was assayed by freezing point depression. Data were statistically analyzed by the method of least significant difference (13).

Treatments. Experiment 1. After an initial control clearance determination, each bird was injected sc with *d,l*-isoproterenol hydrochloride or isotonic saline in a 1-ml volume. After the injection, clearances were determined sequentially three times at 30-

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min intervals. The injectate was varied so that half the birds received saline first and half received isoproterenol first. On another day, the treatment was reversed and clearance measurements were repeated. Hence, the effects of saline and of isoproterenol were measured in each bird.

Experiment 2. Four of the birds were studied further (technical problems precluded study of the other two). Two birds were used as saline-injected controls and two were injected with isoproterenol. The same protocol was used as in experiment 1 except that the carotid artery was cannulated with Clay Adams PE 160 polyethylene tubing under local anesthesia, and systolic, diastolic, mean, and pulse pressures were recorded using a Narco Bio-Systems polygraph and a P-1000 pressure transducer. Two 30-min control periods preceded administration of *d,l*-isoproterenol hydrochloride (400 μg/kg calculated as salt). Afterwards, measurements were made and averaged in each of the three 30-min intervals.

Results. Experiment 1. Isoproterenol, at a dose of 400 μg/kg body wt stimulated urine production (Table I). This effect occurred consistently and began within 10 min of the injection, reached a maximum within 1 hr, and then gradually declined though remaining above control levels until the experiment ended. In preliminary experiments on other birds 180 μg of *d,l*-isoproterenol hydrochloride/kg body wt stimulated urine production in only one of three trials, and 90 μg/kg stimulated urine flow moderately in one trial. Since 400 μg of isoproterenol/kg body wt consistently induced diuresis, the present experiments were performed using this dosage.

The C_{Cr} increased significantly above the level of saline-treated controls after administration of 400 μg of *d,l*-isoproterenol/kg body wt/(Table I). The C_{PAH} in both control and isoproterenol-treated birds decreased during the treatment period.

Both C_{Na} and C_{Osm} increased significantly above the levels of saline-treated controls (Table I). The C_{H_2O} was negative for saline-treated turkeys throughout, but became positive in the first clearance period following administration of isoproterenol and then was highly variable (Table I).

Mean carotid arterial pressure declined in both groups even during the control period, but declined more drastically after administration of isoproterenol (Table II). Mean carotid pressure then stabilized at approximately 60 mm Hg while that of saline-injected birds declined progressively. The de-

TABLE I. RENAL FUNCTION IN TURKEYS INJECTED SC WITH 400 μg OF *d,l*-ISOPROTERENOL HYDROCHLORIDE/kg BODY WEIGHT.[a]

Injectate	Preinjection Control	Postinjection (min) 30	60	90
Urine volume (μl/kg/min)				
Saline	31 ± 4	27 ± 2	25 ± 2	25 ± 2
Isoproterenol	36 ± 4	218 ± 18*,**	352 ± 38*,**	263 ± 38*,**
Creatinine clearance (ml/kg/min)				
Saline	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
Isoproterenol	1.8 ± 0.1	2.5 ± 0.1*,**	2.5 ± 0.1*,**	2.4 ± 0.1*,**
p-Aminohippuric acid clearance (ml/kg/min)				
Saline	28 ± 3	24 ± 3	22 ± 2*	19 ± 2*
Isoproterenol	32 ± 3	24 ± 1*	22 ± 2*	17 ± 1*
Sodium clearance (μl/kg/min)				
Saline	18 ± 5 (5)	19 ± 3	24 ± 2	27 ± 6
Isoproterenol	30 ± 3*,**	174 ± 18*,**	318 ± 27*,**	228 ± 36*
Osmolal clearance (μl/kg/min)				
Saline	61 ± 7 (4)	50 ± 5 (5)	57 ± 5 (4)	44 ± 7 (4)
Isoproterenol	60 ± 4 (4)	196 ± 16 ± (5)*,**	394 ± 41*,**	248 ± 29*,**
Free water clearance (μl/kg/min)				
Saline	−31 ± 4 (4)	−24 ± 6 (5)	−33 ± 4 (4)	−20 ± 6 (4)
Isoproterenol	−24 ± 3 (4)	+34 ± 8 (5)*,**	−40 ± 28	+14 ± 20

[a] Mean ± SE. $N = 6$ unless indicated differently in parenthesis. These differences result from insufficient sample volumes.

* Significantly different from preinjection control ($P < 0.01$).

** Significantly different from saline injected birds ($P < 0.01$).

TABLE II. SYSTEMIC CIRCULATORY CHANGES IN TURKEYS INJECTED SC WITH 400 μg OF *d,l*-ISOPROTERENOL HYDROCHLORIDE/kg BODY WEIGHT.[a]

	Preinjection control (min)		Postinjection (min)		
	30	60	30	60	90
Mean carotid pressure (mm Hg)					
Saline	180	170	161	148	142
Isoproterenol	188	134	60	58	58
Carotid pulse pressure (mm Hg)					
Saline	59	55	45	34	27
Isoproterenol	71	53	67	60	56
Carotid pulse rate (beats/min)					
Saline	225	209	209	204	200
Isoproterenol	183	183	228	230	222

[a] Mean of two saline-injected and two isoproterenol-injected birds. Each value represents mean of measurements made at 5-min intervals over 30 min.

cline in blood pressure of saline-treated birds may have been related to prolonged restraint and/or volume depletion due to blood sampling.

In controls, pulse pressures declined more or less progressively. This did not occur after isoproterenol because reductions in diastolic pressure were proportionately larger than those of systolic pressure. The pulse rate increased moderately after isoproterenol.

Discussion. In turkeys, isoproterenol induced a diuresis in contrast to the antidiuresis reported for certain mammals (1–7). This effect in turkeys might result from direct effects on the kidneys, e.g., altered renal hemodynamics, nephron recruitment, or changes in tubular reabsorptive mechanisms, or it might result from indirect renal effects, e.g., decreased ADH secretion or alterations in the systemic circulation.

Apparently the effects of isoproterenol on the systemic circulation are similar to those described for mammals, i.e., increased myocardial contractility and decreased peripheral vascular resistance (14). Reduced vascular resistance was indicated by the reduction in mean arterial pressure, and by a proportionately larger decrease in diastolic than in systolic pressure. Increased pulse pressure may indicate a greater myocardial contractility leading to an increase in stroke volume. Together with the increased pulse rate, this is indicative of increased cardiac output (15).

The C_{Na} and C_{Osm} consistently increased after isoproterenol. This could result from either increased filtered loads or decreased tubular reabsorption of Na and other osmotically active solutes. Since C_{Cr} increased and Na concentration in plasma usually increased, it is likely that increases in filtered load account for the increases in C_{Na} and C_{Osm} and suggest the diuresis was at least partly osmotic in origin. Such increases in filtered loads could result either from increased filtration rates of individual nephrons, or from recruitment of additional nephrons.

After isoproterenol, C_{H_2O} increased initially and then became highly variable. Concomitantly the urine osmolality fell, the greatest reduction being in the first clearance period. Birds possess two nephron populations, a nonconcentrating reptilian type and a concentrating mammalian type (16). Hence, the C_{H_2O} changes are interpretable in terms of increased reptilian nephron activity, due either to recruitment of more nephrons or to increased filtration by individual nephrons. The C_{H_2O}, however, often is used as an indirect measure of ADH activity. Conceivably, the initial increase in C_{H_2O} resulted from ADH inhibition. Such an effect would, however, be in striking contrast with the effect of isoproterenol in mammals, i.e., increased ADH activity (6–11). Moreover, the isoproterenol-induced hypotension, the diuresis-induced volume depletion, and increased plasma osmolality are likely

to have stimulated ADH secretion.

Creatinine is cleared by glomerular filtration and by renal tubular secretion in birds (17). However, the measured increase in C_{Cr} is likely to reflect primarily increased glomerular filtration. Plasma creatinine concentration, though elevated from normal, consistently decreased with time. Under such conditions, creatinine cleared by tubular secretion would be expected to remain unchanged or decline due to the saturation kinetics of the creatinine secretory mechanism (17). The increments in C_{Cr}, therefore, probably reflect increases in GFR. Histologic evidence, viz, arteriolar and tubular dilatation, supports such an interpretation (1).

Such changes in C_{Cr} could result from reduced resistance (dilatation) in either the afferent arteriole or glomerular capillaries, increased resistance (constriction) in the efferent arteriole, or dilatation of Bowman's capsule or the tubule, thus increasing the glomerular-capsule hydrostatic pressure gradient which drives filtration. This latter effect cannot be ruled out, but there is little precedence for such an effect. More likely, there are preglomerular or glomerular β-receptors which are responsible for decreased vascular resistance and increased glomerular flow due to recruitment of more glomeruli and/or increased filtration by individual glomeruli. Constriction of the vessels is not an affect usually associated with β-adrenergic stimulation, and it is therefore unlikely that there was increased efferent arteriolar resistance.

Summary. The isoproterenol-induced diuresis in turkeys is associated with an increase in C_{Cr} and C_{H_2O}. The effect is probably mediated, in part, by β-adrenergic receptors in the afferent arteriole which lead either to recruitment of reptilian-type nephrons or more filtration by individual nephrons. In other respects the effects of isoproterenol in turkeys resembles those known to occur in mammals.

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