Effects of Bethanechol (Urecholine) on Renal Electrolyte Excretion in Rats and Chickens[1] (34591)

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Several cholinomimetic agents have been reported to produce saluresis or diuresis. In rats, arecoline given subcutaneously produces saluresis (1). Diuresis in dogs is produced when acetylcholine and arecoline are given into the renal artery (2–4, 8). In chickens, acetylcholine and arecoline produce diuresis when infused into the renal portal system (5, 6). Atropine inhibits these renal effects.

Several mechanisms have been proposed to explain the diuretic effects of acetylcholine and cholinomimetics. Since these agents usually produce vasodilatation, an increase in blood flow through the kidney could result in a wash-out of the medullary concentration gradient (7). Some doubt as to the validity of this mechanism for acetylcholine has been presented in the data of Suki *et al.* (8). From results obtained using hydrated dogs and administering acetylcholine into a renal artery these workers concluded that the increased excretion of sodium probably was not due solely to a wash-out effect, since the increased free water clearance indicated a direct tubular effect to decrease sodium reabsorption. It has been postulated that acetylcholine and cholinomimetics could have a direct effect on tubule cells to decrease sodium reabsorption with a resultant diuresis (4, 5, 9, 10).

The experiments presented here were designed using the rat and the chicken, to test whether bethanechol has a diuretic effect which is related to a wash-out of the medullary concentration gradient. In the kidney of the rat marked concentration gradients exist from the papilla to the cortex and therefore the rat is able to concentrate its urine about eight times that of plasma (11). A decrease in these gradients should occur if a wash-out resulted from vasodilatation. The chicken has a rudimentary countercurrent mechanism with only a few long loops of Henle, and the chicken can concentrate its urine only about 1.8 times that of plasma (12). The urine of the chicken is usually hypotonic to plasma (13). Production of a unilateral diuresis from an agent infused into the chicken leg vein would be due most probably to a direct inhibitory effect on sodium reabsorption by the renal tubule cells.

Bethanechol [(2-hydroxypropyl) trimethyl ammonium chloride carbamate] might be expected to produce diuresis since it is a cholinomimetic agent. The effects of arecoline on concentration gradients in rat kidneys have not been reported, and since the renal effects of arecoline were known, it was included in these experiments.

Methods and Materials. Male Sprague-Dawley rats, weighing 220–270 g, deprived of food and water for 18 hr, were hydrated with 25 ml/kg of tap water prior to subcutaneous injection of the agents, which were dissolved in normal saline. The dose of arecoline hydrochloride was 2 mg/kg, and the doses of bethanechol chloride were 5 and 10 mg/kg. Normal saline was used for the control group. After treatment each animal was placed in a metabolism cage, and urine was collected in graduated tubes for 2 hr. At the end of the 2 hr, during maximal diuresis, rats were anesthetized with pentobarbital, 45 mg/kg intraperitoneally. After clamping the renal arteries, the kidneys were removed and sections were cut immediately from the papilla, medulla, and cortex. The tissue samples were weighed (wet weight) and dried for at least 4 hr at

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100–110°. The dry weights of the tissues were determined, and the percentage of water was calculated. The tissues were ashed in a muffle furnace at 500° overnight. After appropriate dilutions, analyses for sodium and potassium in the renal tissue and sodium, potassium, chloride, and osmols in the urine were made.

Unanesthetized, White Rock and White Leghorn hens, weighing 2–4 kg, were placed in a special stand, which allowed maximum comfort to the animal with minimal movement. The urine was collected according to the method of Sperber (14). This method consists of cleaning the cloaca, everting the edges of the cloaca after applying a local anesthetic, and suturing rubber foam-covered collecting tubes to the ureteral openings. In order to keep the tubes open and free of uric acid, demineralized water was infused at a rate of 2.35 cc/10 min through a needle inserted into the rubber collecting tubes. The net urine volume was found as the difference between the total amount of fluid collected and the amount of demineralized water that was added. After the urine flow had stabilized at about 0.6 cc/10 min, urine from each ureter was collected in graduated centrifuge tubes at 10-min intervals, and the volumes of urine were recorded.

The drugs were administered through a needle inserted into the saphenous vein. There was a constant infusion at the rate of 3.88 cc/10 min into the vein of a 0.42 % sodium chloride solution containing sufficient para-aminohippurate (PAH) so that 25 μg/kg/min of PAH was infused. The drugs were dissolved in the sodium chloride solution containing PAH, and 10 and 20 μg/kg/min of bethanechol chloride and 10 μg/kg/min of atropine sulfate were administered. The saline solution containing PAH was infused for at least two initial control periods, and then the drug solutions were infused for at least two periods, following by a minimum of two control periods again before another drug was introduced or the experiment was ended.

The urine samples were analyzed for PAH, sodium, potassium, and chloride. Analyses for sodium and potassium of urine and tissue

TABLE I. Effects of Arecoline and Bethanechol on the Concentration Gradients of the Rat Kidney.

Parameters	Papilla		Medulla		Cortex	
	Control	Exp.	Control	Exp.	Control	Exp.
Exp. 1: Five rats treated with 0.9% NaCl = Control; 5 rats treated with 2 mg/kg arecoline = Exp.						
Water (%)	85 ± 0.4[a]	86 ± 0.6	81 ± 0.5	80 ± 1.0	77 ± 0.2	78 ± 0.3[b]
Sodium[c]	1060 ± 28.4	1379 ±246	418 ± 27.5	419 ± 32.7	232 ± 4.5	253 ± 5.3[b]
Potassium[c]	471 ± 18.3	529 ± 39.5	387 ± 6.5	383 ± 12.1	327 ± 2.0	346 ± 11.0
Exp. 2: Four rats treated with 0.5% sodium chloride; 4 rats treated with 5 mg/kg bethanechol						
Water (%)	86.5 ± .44	85.7 ± .92	81.0 ± .63	81.3 ± 0.42	76.6 ± 0.42	77.6 ± 0.76
Sodium	1016 ± 52	1098 ± 67	435 ± 16	484 ± 26	228 ± 4	259 ± 29
Potassium	417 ± 20	407 ± 43	356 ± 11	367 ± 16	294 ± 16	312 ± 21
Exp. 3: Three rats treated with 0.9% sodium chloride; 3 rats treated with 10 mg/kg bethanechol						
Water (%)	87 ± 0.6	88.0 ± 0.1[b]	80 ± 0.7	82 ± 0.3[b]	77 ± 0.2	78 ± 0.8
Sodium	976 ±104	1020 ±114	406 ± 37.7	434 ± 16.7	219 ± 3.0	223 ± 19.9
Potassium	416 ± 37.3	457 ± 15	342 ± 8.1	386 ± 5.9[b]	308 ± 5.5	311 ± 7.8

[a] Means ± SEM.
[b] $p < .05$, experimental compared to control.
[c] mEq/kg dry wt.

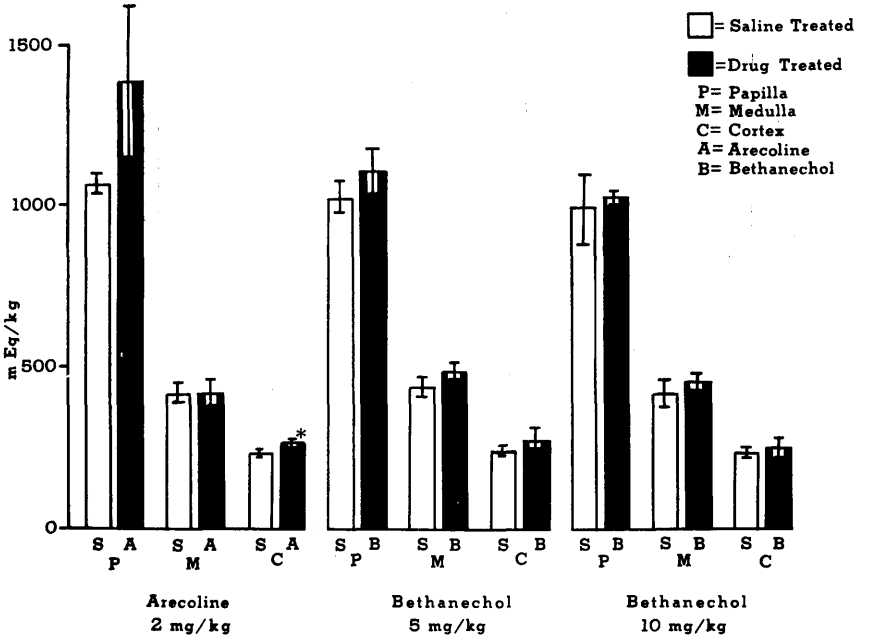

FIG. 1. Sodium concentration in renal papilla, medulla, and cortex of rats treated with saline or either arecoline or bethanechol. Kidneys were removed from animals sacrificed during maximum diuresis of the drugs, *i.e.*, 2 hr after treatment. (* = $p < .05$)

samples were performed using a Baird Flame Photometer. Chloride analyses were made using a Buchler-Cotlove chloridometer. PAH determinations were carried out using the method of Smith *et al.* (15). The osmolalities of rat urine were determined with a Fiske osmometer.

The ATEF or apparent tubular excretion fraction was determined for PAH to assess whether the renal portal system was functioning properly. The ATEF is calculated as follows: (expressed as a percentage)

$$\text{ATEF} = \frac{\text{Rate of PAH excretion from infused side} - \text{Rate of PAH excretion from noninfused side}}{\text{Rate of PAH infusion}}$$

A high ATEF usually indicates that the valve situated at the junction of the iliac and renal veins is contracted and that most of the blood is diverted primarily through the ipsilateral peritubular capillary network. A low ATEF value could indicate relaxation of the valve and diversion of more blood through the renal vein into the general circulation, or it could indicate an inhibition of PAH transport.

The Student t test was used in the statistical analysis of the data (16). A probability level of $p \leqq .05$ was taken as significant.

Results. In Table I and Figs. 1 and 2 are presented data from the rat experiments. In Table I are summarized the data for effects of arecoline and bethanechol on the concentration gradients of sodium and potassium, and the percentage of water in each area of the kidney. Figure 1 depicts graphically the sodium in the three areas of the kidneys of saline- and drug-treated rats. Figure 2 summarizes the urinary excretion data obtained with bethanechol treatment compared to saline treatment.

Data from Expt. 1 (Table I) in which 2 mg/kg of arecoline was used indicate there was no decrease in concentration gradients of the arecoline-treated rats compared to control rats. There were decreasing concentrations of water, sodium, and potassium from the papilla to the cortex in both experimental and control rats' kidneys. Sodium and water were significantly increased in the kidney cortex of the arecoline-treated rats compared with that of control rats. It had been demonstrated previously that arecoline in doses of

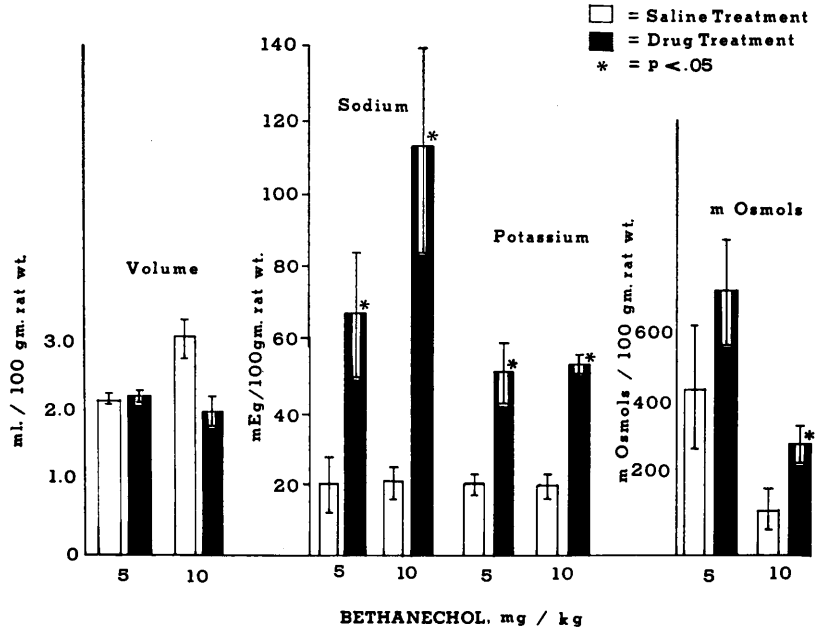

FIG. 2. Urinary excretion data of rats treated with saline or bethanechol. Urine collected for 2 hr after treatment. Values for four saline-treated rats are compared with those for four rats treated with 5 mg/kg of bethanechol; and values for three saline-treated rats compared with three treated with 10 mg/kg bethanechol.

1.25–3.0 mg/kg given subcutaneously to rats produces a significant increase in urinary sodium and chloride excretion (1).

Data from Expt. 2 (Table I) in which 5 mg/kg of bethanechol was used, indicate there were the expected gradients in water, sodium, and potassium content from papilla to cortex with no decrease in the experimental group compared to the control group.

In Expt. 3 (Table I) in which 10 mg/kg of bethanechol was used, there was a significant increase in water in the papilla and medulla, and in potassium in the medulla of the bethanechol-treated rats compared to the control rats.

Depicted in Fig. 2 is the significant increase in the urinary excretion of sodium and potassium at the lower dose and an increase in sodium, potassium, and milliosmols at the 10 mg/kg dose.

Figure 3 portrays results of a typical chicken experiment showing data for sodium excretion, urine volume, and PAH ATEF. In Table II a summary of results from all the chicken experiments is given. Data in this table are expressed as the mean difference between the experimental and control periods for the infused and the noninfused side. The experimental periods were during infusion of the drug, and the control periods were during the infusion of saline solution. The number of experiments are the number of hens used for that particular drug.

During bethanechol infusion, urine volume and sodium excretion were increased on the infused side but not on the noninfused side. The chloride excretion increased from both kidneys at the lower dose level. Potassium excretion was increased only on the infused side. The significance of the increase in PAH ATEF at the 10 μg/kg/min dose is probably not meaningful since an increase of equal magnitude at the 20 μg/kg/min dose was not a significant change. The variability of the PAH ATEF data probably preclude any interpretation of changes.

In three experiments (Table II), infusion of atropine sulfate (10 μg/kg/min) was followed by the infusion of bethanechol (20 μg/kg/min). With atropine infusion there

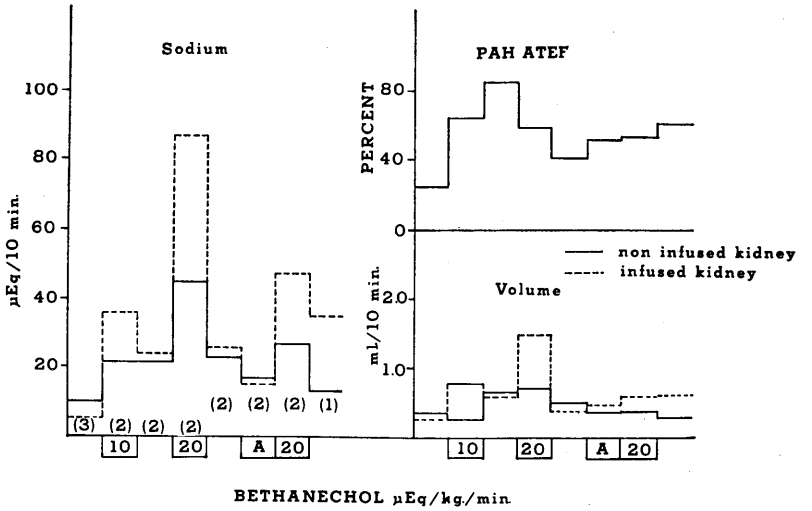

FIG. 3. Typical chicken clearance depicting unilateral increase in sodium, volume, and changes in PAH ATEF during 10-min administrations of 10 and 20 µg/kg/min of bethanechol. In the parentheses are the numbers of 10-min clearances averaged to give sodium values indicated. These numbers also apply to volume and ATEF's. Atropine (10 µg/kg/min) was given for 10 min during interval indicated (A).

were no significant changes in the urine volumes or electrolyte excretion, although there was a tendency for a decrease to occur. The average PAH ATEF seemed to be decreased, but this change was not significant. Rennick and Gandia found that atropine relaxed the valve and this could account for a tendency to have a lower ATEF for PAH (17). The infusion of bethanechol (20 µg/kg/min) after atropine produced no significant change in urine volume, or sodium and chloride excretion. Potassium excretion was significantly increased with bethanechol after atropine on the noninfused side. The apparent increase in PAH ATEF was not significant.

Discussion. Bethanechol produces saluresis

TABLE II. Renal Effects of Bethanechol Chloride in the Chicken.

	Bethanechol (µg/kg/min)				Atropine 10 µg/kg/min		Bethanechol[e] 20 µg/kg/min	
	10		20					
	I[a]	NI	I	NI	I	NI	I	NI
Number of experiments[b]	6		6		3		3	
Urine vol (ml/10 min)	0.37[c]	0.08	0.81[c]	0.08	—0.14	—0.13	0.27	0.083
Sodium (µeq/10 min)	41.2[c]	13.8	66.3[c]	13.9	—27.8	—24.2	27.3	15.8
Potassium (µeq/10 min)	7.2[c]	3.3	9.8[c]	4.5	—5.7	—6.5	12.3	9.8[c]
Chloride (µeq/10 min)	46.3[c]	24.7[c]	56.2[c]	9.0	—32.2	—25.2	43.8	22.7
PAH ATEF[d] (%)	65[c]	42	87	64	22	46	62	22

[a] I = infused side; NI = noninfused side.

[b] The number of experiments are the number of hens used for each dose. There were two to four collection periods per experiment for each dose level and the interspersed control periods.

[c] Significance of $p < .05$. These data represent the mean differences between effects obtained during drug infusion and the preceding control periods.

[d] See text for calculations. The values under the I column represent ATEF's during infusion of the drugs; the values under the NI column represent ATEF's during preceding control periods.

[e] After atropine.

in the rat and diuresis in the chicken. If cholinomimetic agents produce saluresis by vasodilatation and a subsequent wash-out of the concentration gradients of the kidney, one would expect to find a decrease in sodium concentration gradients and an increase in urine volumes. There was no decrease in the concentration gradients of sodium or potassium in the kidneys of rats treated with bethanechol and then sacrificed at the height of the saluretic effect. There was also no change in urine volume. In fact, the data indicate there was an increase in the concentration of sodium and water in the cortex of the experimental animals compared to control animals when arecoline was used. Since the agents used in the rats were given systemically, any release of antidiuretic hormone and catecholamines might oppose vasodilatation and cause antidiuresis. Such an effect is suggested by the decreased urine volume found in the experimental group compared to the control group when a 10 mg/kg dose of bethanechol was used (Fig. 2).

The unilateral diuretic effect produced in the chicken indicates that bethanechol was perfusing the ipsilateral peritubular capillary bed before reaching the systemic circulation. If the smooth muscle valve at the junction of the external iliac vein and the renal vein is contracted, blood is diverted primarily through the external iliac vein and perfuses the peritubular capillaries of the ipsilateral kidney before reaching the systemic circulation. Therefore, a drug which had an ipsilateral effect would be exerting a direct renal effect and not a systemic effect. Any direct effect of atropine on PAH transport to account for the suggested decrease in ATEF was discounted by Rennick (17) by the lack of effect of atropine on PAH uptake in chicken kidney slices. The effect of bethanechol on PAH transport in slices is unknown, but there is no effect of arecoline on PAH transport in rat or chicken kidney slices (May, D.G., and Carter, M.K., unpublished observations). The inhibition of the effects of bethanechol in chickens by atropine indicates that the diuresis from bethanechol is mediated probably through a muscarinic-type receptor.

The effects of bethanechol (5 and 10 μg/kg/min) infused for 10-min periods into the renal artery of a dog, prepared for renal clearances, were a marked unilateral increase in sodium and chloride excretion and urine volume. Potassium excretion and GFR changed very little, but ERPF (PAH clearance) increased moderately. These results are consistent with effects of acetylcholine and arecoline in the dog (2, 3).

May and Carter (5) hypothesized that arecoline in chickens acts by a direct effect on the peritubular side of the tubular cells to increase the permeability of the peritubular membrane to sodium. An increase in influx of sodium could decrease the gradient for sodium from the intraluminal side and result in an increased excretion of sodium. Williams and co-workers (3) suggested that arecoline has a direct renal tubular effect in dogs resulting in an increase in sodium excretion. Carter and Pearson (9) also suggested that cholinomimetic agents might increase the permeability of renal tubular cells, and Parmelee and Carter (6) found that acetylcholine infused into a saphenous vein of the hen produced a unilateral diuresis. They concluded that the action of acetylcholine could be due to a direct tubular effect in this species.

It is suggested that the most probable mechanism of bethanechol to produce a diuresis in the chicken and a saluresis in the rat is a direct tubular effect to decrease sodium reabsorption, and that in the dog this direct effect is in addition to any effects resulting from vasodilatation.

Summary. The effects of bethanechol chloride (5 and 10 mg/kg) and arecoline hydrochloride (2 mg/kg) on the concentration gradients of sodium and potassium were determined in renal papilla, medulla, and cortex of rats sacrificed at the height of the saluresis produced by the subcutaneous injection of these agents. Bethanechol produced an increased urinary excretion of sodium and potassium and an increased osmolality of the urine with no change in volume. Arecoline had been reported previously to have similar effects in rats. The concentration gradients of

sodium and potassium in the kidneys of the rats treated with bethanechol or arecoline were not decreased compared to control rat kidneys.

In the chicken, bethanechol chloride (10 and 20 μg/kg/min) infused into the saphenous vein produced a unilateral increase in urinary volume and an increase in excretion of sodium, potassium, and chloride. Similar effects were noted in the dog when bethanechol was given into one renal artery. These effects were inhibited by atropine.

Since the chicken kidney concentrates urine very little, and since there was no decrease in concentration gradients in kidneys of rats at the height of the saluresis, it is concluded that bethanechol exerts its diuretic or saluretic effects in these species primarily by a direct inhibitory effect on tubular reabsorption of sodium.

The competent technical assistance of Mrs. Betty Maddux is gratefully acknowledged.

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Received July 24, 1969. P.S.E.B.M., 1970, Vol. 133.