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Effect of Cholera Toxin on Renal Tubular Reabsorption of Glucose and Bicarbonate (40281)[1]

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Cholera toxin (CT) produces fluid and electrolyte secretion in the small intestine due to stimulation of adenylate cyclase and increased production of adenosine 3′5′ cyclic monosphosphate (cAMP) (1–6). Other studies have shown that the adenylate cyclase–cyclic AMP system is stimulated by CT in a variety of tissues such as liver, thyroid, adrenal, fat and leukocytes with no demonstration of any other major structural or enzymatic changes (7–13). Thus, CT may provide a pharmacologic tool for the study of the effects of stimulating adenylate cyclase–cyclic AMP systems (10, 11).

We have previously shown that the infusion of cholera toxin into one renal artery of dogs is followed by decreased net tubular reabsorption of sodium, potassium, calcium, magnesium and phosphate with interrelationships similar to those observed during expansion of the extracellular fluid volume with saline (14). Further studies from our laboratory have demonstrated that expansion of the extracellular fluid with a Ringer bicarbonate solution is accompanied by increased net production of cyclic AMP by the kidney suggesting a role for cyclic AMP in the reabsorption of these various ions (15).

Since extracellular fluid volume expansion is accompanied by decreased tubular reabsorption of glucose (16, 17) and bicarbonate (18–20) due to suppression in their reabsorption, which occurs mostly in the proximal tubule (20–25), this study was designed to evaluate whether the stimulation of renal adenylate cyclase with CT also affects the reabsorption of these two substances in an effort to further document a relationship between renal cAMP and tubular reabsorptive processes.

Material and methods. Twelve experiments were carried out in female mongrel dogs weighing from 18 to 27 kg, anesthetized with pentobarbital (30 mg/kg). The dogs were ventilated through a cuffed endotracheal tube with a Harvard Respirator. Both ureters were cannulated through bilateral flank incisions and a curved 23 gauge needle was placed in the left renal artery in the direction of blood flow. Isotonic saline was infused into the renal artery at a rate of 1 ml per min throughout the studies. A catheter was placed in the aorta through a femoral artery to obtain blood samples and to measure arterial pressure with an aneroid manometer. All experiments were started at least 60 min after completion of surgery. Glomerular filtration rate (GFR) was measured using the clearance of exogenous creatinine with standard priming dose and constant infusion technique. Urine collections of 10 min duration were obtained throughout the studies with blood obtained at the midpoint of each period. After 3–4 control periods purified cholera toxin (Schwarz-Mann, Orangeburg, NY) was added to the renal arterial infusion to deliver 8 μg/min for 180 min.

The effect of CT on glucose reabsorption was evaluated in five dogs. An intravenous solution containing glucose (10–15%), sodium (23 mEq/l), potassium (10 mEq/l) and chloride (33 mEq/l) was given at a rate of 8 ml/min in order to attain a stable high level of blood glucose at the time of the maximal effect of CT on tubular transport of electrolytes which usually occurs 100–140 min after the administration of CT (14). Both blood and urine samples were collected in ice cold

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test tubes and triplicate determinations of glucose were performed.

The effect of CT on bicarbonate reabsorption was studied in seven dogs. In order to raise the blood bicarbonate to a stable level of 33–37 mEq/l, the animals received pulse injections of bicarbonate 50–90 mEq at the beginning of the study and every 40 min thereafter and a constant infusion of a solution containing bicarbonate (240 mEq/l), sodium (263 mEq/l), potassium (10 mEq/l), and chloride (33 mEq/l) at a rate of 4 ml/min. The rate of respiration was adjusted by the Harvard Respirator to keep PCO_2 stable around 40 mm Hg. Urine was collected anaerobically under mineral oil from the ureteral catheters and blood samples were obtained anaerobically in syringes containing heparin.

These protocols allowed us to compare tubular reabsorption of glucose (TRG) and bicarbonate ($TRHCO_3$) by both kidneys when all variables other than the infusion of CT into one renal artery were equal.

The concentration of creatinine in the blood and urine samples were determined with Technicon autoanalyzer (Tarrytown, NY), sodium and potassium with Instrumentation Laboratory flame photomoter (Lexington, MA), chloride with CMT 10 chloridometer (Radiometer, Copenhagen), glucose with Beckman glucose analyzer (Beckman Instruments Incorporated, Palo Alto, California) which utilizes glucose oxidase (26), and pH and PCO_2 with a Radiometer acid base analyzer, Model BMS 3-PHM71 (Radiometer, Copenhagen). The concentration of bicarbonate in plasma and urine were calculated from the Henderson-Hasselbach equation utilizing the following factors: Solubility coefficient for CO_2 in plasma and urine of 0.0301 and 0.0309, respectively; a pk of 6.10 for plasma and a pk for urine calculated from its ionic strength according to the formula, $pKa = 6.33 - 0.5\sqrt{Na^+ + K^+}$ with the concentrations of Na and K given in equivalents per liter (27). Paired data analysis was used to evaluate the statistical significance of the results which are expressed as mean ± SEM.

Results. Effect of CT on glucose reabsorption (TRG). The effect of the infusion of CT on GFR, fractional excretion of sodium (FE_{Na}) and glucose reabsorption are given in Table I and Fig. 1. There were no significant differences among these parameters between both kidneys prior to the infusion of glucose and CT. Renal TRG after 100–140 min of CT was 80.1 ± 20.2 mg/min, a value significantly ($P < .05$) lower than that observed for the contralateral kidney (98.7 ± 20.7 mg/min). Renal TRG per 100 ml GFR was 254 ± 32.7 mg, a value significantly ($P < .01$) lower than that observed in the opposite kidney (363 ± 43.5 mg per 100 ml GFR). The FE_{Na} increased significantly from both kidneys but it was markedly higher ($P < .01$) from the kidney receiving CT (11.2 ± 2.82%) than the contralateral kidney (4.62 ± 1.42%).

The values for TRG per 100 ml GFR in all measurements made from both kidneys during the period of 100–140 min after the initiation of the infusion of CT and when filtered glucose ranged between 700–1900 mg per 100 ml GFR are shown in Fig. 1. For any given level of filtered glucose, TRG per 100 ml GFR was lower in the kidney infused with CT.

Effect of CT on bicarbonate reabsorption. The effects of CT infusion on GFR, FE_{Na}, $TRHCO_3$/GFR and the urinary excretion of sodium, chloride and bicarbonate are given in Table II and Figs. 2 and 3. Again, there were no significant differences between these parameters prior to the infusion of bicarbonate and CT. Renal $TRHCO_3$ after 100–140 min of CT was not different between both kidneys while $TRHCO_3/GFR \times 100$ by the infused kidney was 2.09 ± .06 mEq per 100 ml GFR, a value significantly lower ($P < .01$) than that observed in the contralateral kidney (2.53 ± .06 mEq per 100 ml GFR). Figure 2 provides data on $TRHCO_3$/GFR for all measurements obtained during the maximal effect of CT and a filtered bicarbonate ranging between 2.8 to 4.1 mEq per 100 ml GFR. Again, $TRHCO_3/GFR \times 100$ for any given level of filtered carbonate was lower under the effect of CT.

The FE_{Na} increased in both kidneys but was significantly higher ($P < .01$) in the kidney receiving CT (15.9 ± 0.74%) than that of the contralateral kidney (7.1 ± .26%). The increments in urinary sodium in the CT kidney were due to both NaCl diuresis (40%) and $NaHCO_3$ excretion (60%) while the ex-

TABLE I. EFFECTS OF CHOLERA TOXIN ON RENAL TUBULAR REABSORPTION OF GLUCOSE.[a]

Experiment	C_{Cr} ml/min		$C_{Na}/C_{Cr} \times 100$ %		PG	TRG mg/min		$TRG/C_{Cr} \times 100$ mg	
	L	R	L	R	mg/dl	L	R	L	R
1. Control	14.5	19.8	0.06	0.04	161	23.0	32.0	161.0	161.0
CT + glucose	23.0	23.7	6.50	1.23	993	65.9	84.5	285.3	355.6
2. Control	24.6	26.1	0.50	0.70	142	35.0	48.0	142.0	142.0
CT + glucose	21.1	16.6	16.20	8.50	975	26.0	33.0	123.6	197.4
3. Control	39.4	39.6	0.14	0.19	156	61.0	61.5	156.0	156.0
CT + glucose	51.6	38.2	14.70	1.41	1027	149.4	161.1	288.0	423.0
4. Control	37.8	37.0	0.99	0.27	124	46.7	45.6	124.0	124.0
CT + glucose	24.2	24.5	9.50	6.20	1570	68.8	106.3	283.0	433.0
5. Control	38.8	36.0	0.73	0.67	164	63.6	58.9	164.0	164.0
CT + glucose	29.3	26.6	9.13	5.77	1553	90.3	108.7	292.0	406.0
Control, mean	31.0	31.7	0.48	0.37	149.4	45.9	49.2	149.4	149.4
SEM	4.95	3.75	0.17	0.13	7.38	7.70	5.27	7.38	7.38
CT + glucose, mean	29.8	25.9	11.20	4.62	1123.6	80.1	98.7	254.4	363.0
SEM	5.61	3.50	1.82	1.42	138.2	20.2	20.7	32.7	43.5
P									
L vs R									
Control	NS		NS			NS		NS	
CT + glucose	NS		<0.01			<0.05		<0.01	
P									
Control vs CT + glucose	NS	NS	<0.01	<0.05	<0.01				

[a] Each point represents the mean of three to five consecutive collections. The results obtained during cholera toxin (CT) and glucose infusion represent the mean of three to five consecutive 10 min collections during the maximum response to CT and stable high plasma glucose. C_{Cr} = clearance of exogenous creatinine. $C_{Na}/C_{Cr} \times 100$ = fraction of filtered sodium excreted. PG = plasma glucose. $TRG/C_{Cr} \times 100$ = renal tubular reabsorption of glucose per 100 ml of glomerular filtration. L = left kidney infused with cholera toxin 8 μg per min; R = right noninfused kidney; Control = collections obtained of prior to the infusion of cholera toxin and glucose. CT + glucose = collections obtained at peak effects of cholera toxin (100–180 min) and stable levels of high plasma glucose.

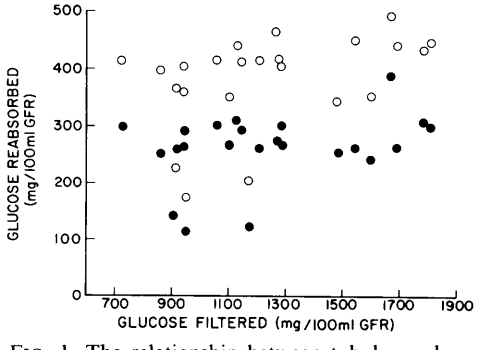

FIG. 1. The relationship between tubular reabsorption of glucose and filtered load of glucose in dogs receiving cholera toxin into the left renal artery. Data from the left kidney receiving cholera toxin infusion are shown in black dots and from the right kidney not receiving cholera toxin infusion are presented in open circles. Data is expressed as mg per 100 ml GFR.

cretion of NaCl comprised only 8% of urinary sodium from the contralateral kidney with the rest (92%) being $NaHCO_3$ (Fig. 3).

Discussion. The results of the present study demonstrate that the infusion of CT into one renal artery is accompanied by a decrease in the renal tubular reabsorption of both glucose and bicarbonate by the infused kidney.

Changes in glomerular filtration rate and alterations in status of extracellular fluid volume (ECF) are known to influence tubular reabsorption of glucose (16, 17, 20, 28, 29). Thus, when the absolute amount of glucose reabsorbed is plotted against GFR in animals in which the rates of sodium reabsorption were unchanged, a direct linear relationship was found (17). In our studies, GFR was either unchanged or modestly increased in the infused kidney at a time when TRG was lower. This observation clearly excludes changes in GFR as the cause for the reduced TRG by CT.

During expansion of ECF, there is an inverse relationship between tubular reabsorption of glucose per unit GFR and the fraction of filtered sodium excreted (17) suggesting that the mechanism responsible for the tubular reabsorption of glucose and sodium may be related. Furthermore, factors that inhibit renal transport of sodium such as ouabain or acetylstrophantidin suppress the reabsorption of glucose as well (30). Changes in the status of ECF could not account for

TABLE II. EFFECTS OF CHOLERA TOXIN ON RENAL TUBULAR REABSORPTION OF BICARBONATE.[a]

Experiment	C_{Cr} ml/min L	C_{Cr} ml/min R	$C_{Na}/C_{Cr} \times 100$ % L	$C_{Na}/C_{Cr} \times 100$ % R	$PHCO_3$ mmol/L	$TRHCO_3$ μEq/min L	$TRHCO_3$ μEq/min R	$TRHCO_3/C_{Cr} \times 100$ mEq L	$TRHCO_3/C_{Cr} \times 100$ mEq R
1. Control	24.3	24.9	1.49	1.62	19.0	448	454	1.78	1.80
CT + $NaHCO_3$	41.2	30.0	17.65	6.80	33.0	815	696	1.98	2.31
2. Control	45.8	46.4	0.10	0.09	23.2	1053	1070	2.29	2.31
CT + HCO_3	25.4	26.1	17.40	6.54	34.2	496	646	1.96	2.48
3. Control	26.6	22.9	0.05	0.07	19.2	515	448	1.91	1.91
CT + HCO_3	28.8	26.0	15.05	6.79	33.8	561	646	1.95	2.48
4. Control	32.2	32.1	0.27	0.31	22.5	719	717	2.24	2.24
CT + HCO_3	40.1	34.0	15.97	6.63	35.2	912	873	2.27	2.56
5. Control	37.7	38.7	0.09	0.09	22.7	848	869	2.24	2.24
CT + HCO_3	45.7	45.3	11.90	8.04	37.7	1028	1159	2.25	2.81
6. Control	37.0	36.0	2.21	2.20	19.6	714	695	1.93	1.93
CT + HCO_3	33.1	27.1	16.85	8.17	33.2	659	651	1.98	2.40
7. Control	38.9	32.6	0.12	0.15	19.9	775	651	1.99	1.99
CT + HCO_3	43.2	37.1	16.20	6.69	36.8	965	1001	2.24	2.69
Control, mean	34.6	33.4	0.62	0.65	20.9	724.6	700.6	2.05	2.05
SEM	2.82	3.04	0.33	0.33	0.69	76.6	83.4	0.08	0.08
CT + $NaHCO_3$, mean	36.8	32.2	15.86	7.09	34.8	776.6	810.3	2.09	2.53
SEM	2.92	2.70	0.74	0.26	0.69	78.3	77.8	0.06	0.06
P									
L vs R									
Control	ND		NS		—	NS		NS	
CT + $NaHCO_3$	<0.05		<0.01		—	NS		<0.01	
Control vs CT + $NaHCO_3$	NS	NS	<0.01	<0.05	<0.01				

[a] Each point represents the mean of three to five consecutive collections. The results during cholera toxin and bicarbonate infusion represent the mean of three to five consecutive collections during the peak effect of cholera toxin (100–180 min) and stable high plasma bicarbonate. C_{Cr} = clearance of creatinine; $C_{Na}/Cr \times 100$ = fraction of filtered sodium excreted; $PHCO_3$ = plasma bicarbonate; $TRHCO_3$ = tubular reabsorption of bicarbonate; $TRHCO_3/C_{Cr} \times 100$ = renal tubular reabsorption of bicarbonate per 100 ml of glomerular filtration; L = left kidney infused with cholera toxin, 8 μg per min; R = right noninfused kidney; control = collections obtained prior to the infusion of cholera toxin and bicarboante; CT + HCO_3 = collections obtained during maximum effect of cholera toxin and during stable high levels of serum bicarbonate.

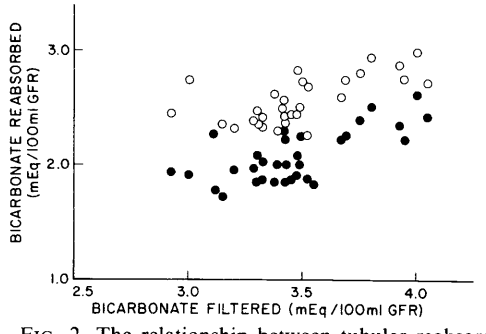

FIG. 2. The relationship between tubular reabsorption of bicarbonate and filtered load of bicarbonate in dogs receiving cholera toxin into the left renal artery. Data from the left kidney receiving cholera toxin infusion are shown in block dots and from the right kidney without infusion are presented in open circles. Data is expressed as mEq per 100 ml GFR.

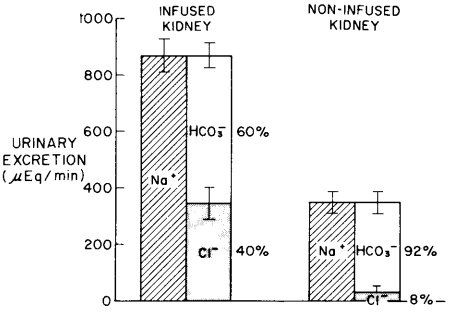

FIG. 3. Urinary excretions of sodium, bicarbonate and chloride during cholera toxin and bicarbonate infusion. Values are the mean and SEM of all experiments during maximal effects of cholera toxin.

our observation since both kidneys were subjected to the same conditions of ECF. However, CT produced a greater degree of natriuresis in the infused kidney and this may directly or indirectly have influenced glucose reabsorption.

The tubular reabsorption of bicarbonate is affected by at least five factors. These include GFR (31), PCO_2 (32, 33), potassium metabolism (34), status of ECF (18, 19), and carbonic anhydrase activity (33). Most of these

factors could not account for the differences in $TRHCO_3/GFR$ from both kidneys. Acute reductions in GFR are associated with a fall in absolute $TRHCO_3$ but $TRHCO_3/GFR$ remains unchanged (31), thus one would anticipate that a rise in GFR should be associated with a proportional increase in $TRHCO_3$ with $TRHCO_3/GFR$ remaining constant. In our bicarbonate infusion studies, the GFR in infused kidney was higher than the control kidney but $TRHCO_3/GFR$ was lower. The blood levels of PCO_2, potassium, as well as the state of ECF could not provide explanation for the unilateral decrease in $TRHCO_3/GFR$ since both kidneys were exposed to the same conditions.

The mechanism(s) through which CT affects glucose and bicarbonate reabsorption are not evident. Several possibilities should be considered. First, CT most probably affects renal tubular transport processes by the stimulation of a renal adenylate cyclase with increased production of cyclic AMP (14, 35). Several lines of evidence exist indicating that cyclic AMP reduces reabsorption of various ions in the proximal tubule (36–38). The studies of Lorentz (39) and Jacobson (46) suggest this effect of cAMP is mediated by an increase in tubular permeability allowing augmented back flux. It is therefore, plausible that the decrease in glucose and bicarbonate reabsorption during CT infusion is secondary to enhanced back flux of reabsorbate produced by cAMP. The observation of Karlinsky *et al.* (41) who showed that the infusion of dibutyryl cyclic AMP reduced the tubular reabsorption of bicarbonate provides further support for the role of CT induced cAMP production in the genesis of reduced $TRHCO_3$.

Second, cholera toxin may directly affect the tubular transport of glucose and bicarbonate. In the ileum, CT enhances bicarbonate secretion (1) but there is no evidence for an effect of CT on glucose transport by the gut (42). Finally, CT may inhibit carbonic anhydrase activity and result in reduced reabsorption of bicarbonate; there is no evidence as yet supporting such a contention.

Most of glucose (21, 24, 25) and bicarbonate (22, 23) reabsorption occur in the proximal tubule. Our present observations of decreased TRG and $TRHCO_3$ and natriuresis are consistent with an effect of CT in the proximal tubule. However, marked decreases in proximal tubular reabsorption of sodium may not be followed by substantial natriuresis unless distal reabsorption of sodium is also reduced (43, 44). It seems, therefore, that CT should have an effect on tubular reabsorption in more distal portions of the nephron as well. Indeed, the observation that during bicarbonate loading 40% of the natriuresis in the infused kidney was due to NaCl as opposed to only 8% in the control noninfused kidney (Fig. 3) suggests that CT may have an effect on tubular reabsorption of Na at more distal sites of the nephron where Na is reabsorbed mostly as NaCl.

The present results together with our previous observations (14) have shown certain analogies between the natriuresis of expansion of ECF and that induced by CT: (a) Both are accompanied by depressed tubular reabsorption of glucose and bicarbonate, phosphate, calcium, magnesium and sodium chloride and (b) the relations between the fraction of filtered Na excreted and that of calcium and magnesium are similar in both conditions. Since extracellular fluid volume expansion with saline is accompanied by increased renal production of cyclic AMP (15) and the renal effects of CT are presumably mediated by stimulation of a renal adenylate cyclase-cyclic AMP system (14, 35), it could be postulated that at least part of the reduction in the tubular reabsorption of these various substances which occur during expansion of ECF may be mediated by increased production of cyclic AMP.

Summary. Cholera toxin (CT) reduces tubular reabsorption of Na, Cl, Ca, Mg and P most probably through stimulation of a renal adenylate cyclase–cyclic AMP system, and it is possible that an increased production of nephrogenous cyclic AMP during extracellular fluid volume expansion may be partly responsible for the observed natriuresis. In order to further evaluate the role of renal cyclic AMP in renal tubular transport, we studied the effect of CT on glucose (TRG) and bicarbonate reabsorption ($TRHCO_3$).

During the period of maximal effect of CT on tubular transport (100–140 min of CT infusion into one renal artery) both the TRG and $TRHCO_3$ were lower in the infused kid-

ney than in the contralateral noninfused kidney; TRG as mg per 100 ml GFR was 254 ± 32.7 vs 363 ± 43.5 ($P < .01$), and $TRHCO_3$ as mEq per 100 ml GFR was 2.09 ± 0.06 vs 2.53 ± 0.06 ($P < .01$). The data indicate that CT suppresses glucose and bicarbonate reabsorption together with that of sodium and as such assign to role for renal cyclic AMP in the regulation of the tubular transport of these substances.

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