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Effect of Diuretics on Intestinal Transport of Electrolytes, Glucose, and Amino Acid[1, 2] (39306)

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The effect of natriuretic agents, ethacrynic acid (EA), furosemide, and amiloride, on ion transport across the renal tubules has been well discussed in the literature (1-4). In terms of site of action and mechanism of action, these agents are different. Recent reports have shown that EA can produce a depressive effect on intestinal transport of electrolytes, especially the sodium ion, resulting in a diarrheal reaction (5-7). Although there is a great similarity in intestinal and renal transport for glucose and amino acids, there has been no clear exploration as to whether or not the electrolyte transport across the intestinal mucosa follows the same pattern as observed in the renal tubules. For example, our previous studies have demonstrated that mice intestine actively reabsorb Na ion and secrete Cl ion (8). Kokko (9) and Burg *et al.* (10) separately reported that there is an active reabsorptive process for Cl ion as well as Na ion in the ascending limb of Henle's loop of the kidney and furosemide exhibits an inhibitory effect on Cl reabsorption. The question is then raised: do these three diuretics affect the electrolyte transport across the mouse intestine? And, if so, what is the mechanism of action?

The *in vitro* experiment with the Ussing Chamber technique has been widely used by many investigators and ourselves to study the ion transport across the mammalian intestine (6, 8, 11, 12, 20). The intention of the present investigation is to utilize this technique for comparative studies of the effect of these three diuretics on the transport of Na and Cl ions. Furthermore, we also intend to show whether these three diuretics affect the glucose and amino acid transport across the intestinal mucosa.

Experimental. Female albino Swiss-Webster mice, averaging 30-40 g in weight, were used. They were kept in a constant temperature room with food and water *ad lib.* for more than a month after purchase. Each mouse was killed by decapitation and the abdomen was opened. Two adjacent sections from the upper jejunum were removed and opened along the mesenteric line, rinsed with Krebs-Ringer solution and mounted in a lucite Ussing chamber as described in our previous paper (8). Mammalian Krebs-Ringer with or without 5.5 m*M* D-glucose was used as a bathing solution. The bathing solutions in both sides of the chamber were continuously oxygenated by bubbling with 95:5% of O_2:CO_2 gas. The chamber was kept at a constant temperature of 37°. The potential difference (PD) across the intestinal mucosal membrane was measured with two calomel electrodes and current was sent to zero out the PD and recorded by an Esterline-Angus recorder. Occasionally the resistance across the membrane was measured and recorded.

For isotopic flux measurements, radioactive ^{22}Na and ^{36}Cl were added into the bathing solution on one side of the membrane, and duplicate samples were collected at 30-min intervals from the other side of the chamber. One sample was counted in an automatic Nuclear-Chicago Well Scintillation counter and the duplicate in a gas-flow Geiger counter. Standard solutions of either ^{22}Na or ^{36}Cl were counted simultaneously with the samples and used for calculating by a computerized program the amounts of ^{22}Na and ^{36}Cl in each sample.

Glucose and amino acid transport. Everted sac technique (13) was used to study the transmucosal transport of D-glucose and L-tyrosine. D-[^{14}C]Glucose (u. l.) and L-[3,5-^{3}H]tyrosine were used as tracers

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for analysis. Both mucosal and serosal fluids were the same Krebs Ringer solution containing 5.5 mmole of D-glucose and 1 mmole of L-tyrosine and the sacs were incubated at 37° for 1 hr under 1 atm pressure of O_2–CO_2 gas. After incubation, the serosal fluid from each sac was removed, centrifuged, and a 50 lambda sample was pipetted into 10 ml of Brays scintillation fluid (14) which was counted in an automatic Liquid Scintillation counter.

Materials. Ethacrynic acid (EA) and amiloride in pure crystalline form were kindly supplied by the Merck Institute for Therapeutic Research. Furosemide in powder form was kindly provided by Hoechst Pharmaceutical Company. All radioactive isotopes were purchased from New England Nuclear Corp. (Boston).

Results. Potential difference (PD) and short circuit current (Isc) measurements. As reported in our previous studies (8), mice intestine mounted in a Ussing chamber with a window area of 1.3 cm^2 exhibited a PD ranging from 1–5 mV with serosa electropositive to mucosa. The short circuit current ranged from 34–42 $\mu A\ cm^{-2}$. Both PD and I_{sc} measurements for each intestinal membrane were quite steady for at least 1.5 hr. When the studied diuretic was added to the serosal bathing medium at concentrations from 0.1–0.5 m*M*, no immediate change of the measured parameters was observed. At higher concentrations, above 0.5 m*M* and especially in the case of ethacrynic acid (EA), the PD and I_{sc} were observed to gradually fall after 15–30 min to about 5–10% of the control level. This effect is probably due to the diffusion of the diuretic from the serosal side into the mucosal side. However, no chemical determination of the diuretic was performed to substantiate such suggestion. When the studied diuretic was added to the mucosal fluid and serosal fluid simultaneously, an immediate but transient rise of PD and I_{sc} followed quickly by a decrease was observed. Such a decrease usually reached a plateau after 15 min and continued thus for 1 to 1.5 hr.

Electrolyte transport. In order to secure a steady and consistent measurement of ion fluxes under the influence of studied diuretics, it is desirable to add the diuretic into bathing medium on both sides of the membrane to avoid the rapid diffusion of the compound from one side to the other. Table I summarizes the simultaneous isotopic flux measurements obtained from two adjacent jejunal sections. Under the condition where the bathing fluid contained 5.5 m*M* D-glucose as substrate, in eight control studies a net Na flux from mucosal-to-serosal side averaging 3.44 $\mu Eq \cdot cm^{-2}\ hr^{-1}$ and a net Cl flux from serosal-to-mucosal side averaging 4.48 $\mu Eq \cdot cm^{-2}\ hr^{-1}$ were obtained. Statistical evaluation of these data indicates that the J_{ms} values of both Na^+ and Cl^- are significantly different from the corresponding J_{sm} values.

In a total of 13 pairs of experiments with EA, both Na and Cl fluxes were significantly inhibited. This inhibitory effect was observed to be more pronounced in the J_{ms} Na flux, resulting in a negative net Na flux which was 130% less than that of control value as compared with the net Cl. flux which was 46% less than that of control value. However, when the bathing medium contained no glucose, seven control studies exhibited average net Na and Cl fluxes of 0.4 and 2.94 $\mu Eq \cdot cm^{-2}\ hr^{-1}$, respectively. In presence of 0.5 m*M* EA, no significant effect was observed in either Na or Cl flux. Table II provides a statistical evaluation of the data.

The other diuretics, furosemide and amiloride, at 0.5 m*M* concentrations, did inhibit both Na and Cl fluxes significantly but were relatively less potent than EA when the experiments were performed with glucose-containing medium. However, when the bathing solution contained no glucose as substrate, both furosemide and amiloride did exert a significant inhibition on Cl flux, 52 and 48%, respectively, but had little effect on Na flux.

Glucose and amino acid transport. Table III presents the data on transmucosal glucose and L-tyrosine transport obtained from the everted intestinal sac technique. At 0.5 m*M* concentration, EA inhibited glucose transport significantly as well as the L-tyrosine transport; but at 1 m*M* concentration such inhibition was markedly significant. The rate of glucose and L-tyrosine transport was reduced to 45% and 55% of the control values, respectively. At a concentration of 1 m*M* and under the same conditions as with

TABLE I. EFFECT OF DIURETICS ON ION TRANSPORT ACROSS THE MICE INTESTINE.

Diuretics (Number of Experiments)	Ion	Flux (μEq·cm^{-2} hr^{-1}) J_{ms}	J_{sm}	J_{net}	P^a	Inhibited[a] (%)	I_{sc}	J^R_{net}[b]
			I. Bathing fluid contained 5.5 m*M* D-glucose					
Control (8)	^{22}Na	18.62 ± 0.74[c]	15.06 ± 0.38	+3.44 ± 0.30				
	^{35}Cl	7.40 ± 0.20	11.88 ± 0.42	−4.48 ± 0.30				
	Total			+7.92			1.38 ± 0.006	−6.54
Ethacrynic acid, 0.5 m*M*(13)	^{22}Na	12.36 ± 0.33	13.44 ± 0.70	−1.08 ± 1.12	<0.01	131		
	^{36}Cl	6.12 ± 0.42	8.50 ± 0.82	−2.38 ± 0.46	<0.01	46		
	Total			+1.30			0.89 ± 0.07	−0.41
Furosemide, 0.5 m*M* (13)	^{22}Na	15.80 ± 0.96	14.40 ± 0.56	+1.40 ± 0.78	<0.01	59		
	^{36}Cl	5.62 ± 0.46	9.22 ± 0.38	−3.06 ± 0.52	<0.01	32		
	Total			+4.46			1.27 ± 0.08	−3.19
Amiloride, 0.5 m*M* (11)	^{22}Na	14.80 ± 0.60	14.16 ± 0.86	+0.66 ± 0.94	<0.01	81		
	^{36}Cl	6.68 ± 0.40	8.34 ± 0.32	−1.66 ± 0.52	<0.01	63		
	Total			+2.32			1.27 ± 0.02	−1.04
			II. Bathing fluid contained *No* D-glucose					
Control (7)	^{22}Na	11.26 ± 0.57	10.83 ± 0.82	+0.40 ± 1.00				
	^{36}Cl	6.70 ± 0.44	9.72 ± 0.60	−2.94 ± 0.58				
	Total			+3.34			0.51 ± 0.02	−2.83
Ethacrynic acid, 0.5, m*M* (13)	^{22}Na	11.62 ± 0.62	11.98 ± 0.60	−0.38 ± 0.56	>0.5			
	^{36}Cl	7.10 ± 0.38	9.52 ± 0.52	−2.42 ± 0.48	>0.5	18		
	Total			+2.04			0.38 ± 0.02	−1.46
Furosemide, 0.5 m*M*	^{22}Na	11.58 ± 0.50	11.10 ± 0.80	+0.48 ± 0.60	>0.5			
	^{36}Cl	5.80 ± 0.46	7.22 ± 0.46	−1.42 ± 0.30	<0.01	52		
	Total			+1.90			0.37 ± 0.035	−1.53
Amiloride, 0.5 m*M* (10)	^{22}Na	10.60 ± 0.48	10.08 ± 0.40	+0.52 ± 0.66	>0.5			
	^{36}Cl	5.68 ± 0.38	7.12 ± 0.52	−1.52 ± 0.60	<0.05	48		
	Total			+2.04			0.34 ± 0.035	−1.70

[a] Compared with the control
[b] $J^R_{net} = I_{sc} - (J^{Na}_{net} - J^{Cl}_{net})$.
[c] Mean ± SEM.

TABLE II. STATISTICAL EVALUATION OF DIURETICS EFFECT ON ION FLUX AS COMPARISON WITH THE CONTROL.

	Bathing Solution			
	With glucose, 5.5 m*M*		Glucose-free	
	J_{ms}	J_{sm}	J_{ms}	J_{sm}
Ethacrynic acid				
Na	$P < 0.01$	<0.01	NS	NS
Cl	$P < 0.01$	<0.01	NS	NS
Furosemide				
Na	$P < 0.01$	NS	NS	NS
Sl	$P < 0.01$	<0.01	NS	<0.01
Amiloride				
Na	$P < 0.01$	NS	NS	NS
Cl	$P < 0.05$	<0.01	<0.05	<0.01

EA, both furosemide and amiloride did not affect either glucose or L-tyrosine transport across the intestinal mucosa.

Discussion. Data presented here demonstrate that the three diuretics, EA, furosemide, and amiloride, reduced both PD and I_{sc} across the mice small intestine. Such a reduction was attributed to the decrease of a net mucosa-to-serosal Na^+ absorption and a serosal-to-mucosal Cl^- secretion. As shown in Table I, when the intestinal mucosa was bathed in Krebs-Ringer solution containing 5.5 m*M* glucose, the inhibitory effect of EA was more prominent in the J_{ms} flux than the J_{sm} flux of Na ion, resulting in a negative net Na flux. With everted sacs of rat intestine, Strombeck and Ingraham (15) found that EA did not inhibit Na^+ transport; however, this was not under short circuit condition. It is difficult to tell whether such refractoriness to EA in rat intestine is a kind of species variation, as observed by its diuretic effect in rat kidney, or is the result of different experimental procedures. Our isotopic flux measurement also demonstrates that EA not only inhibits the net Na flux but also both J_{ms} and J_{sm} fluxes of Cl ion, although slightly, resulting in a decrease in Cl^- secretion and the residual flux (J^R_{net}). If we accept the assumption that the calculated residual flux (J^R_{net}) in the mice intestine is mainly due to the bicarbonate flux (8), then, EA caused the J^R_{net} flux to drop to almost zero aside from its inhibitory effect on Na and Cl fluxes, and such inhibition of HCO_3^- flux can also interfere with water absorption

TABLE III. EFFECT OF DIURETICS ON INTESTINAL TRANSPORT OF D-GLUCOSE AND L-TYROSINE.

Diuretics	Concentration (m*M*)	Expt	Rate of transport (μmol g^{-1} hr^{-1}) D-glucose	Inhibited (%)	L-tyrosine	Inhibited (%)
Control		10	30.60 ± 1.70[a]		2.34 ± 0.17	
EA	0.5	6	24.93 ± 1.49**	19	1.97 ± 0.13	16
	1.0	7	16.80 ± 1.78***	45	1.05 ± 0.19***	55
Furosemide	1.0	7	27.24 ± 3.0	11	1.92 ± 0.23	18
Amiloride	1.0	8	31.58 ± 4.0		2.00 ± 0.31	15

[a] Mean ± SEM.
* $P < 0.05$.
** $P < 0.01$.

causing the diarrheal effect as observed clinically in human patients (7).

Giebisch and his associates (16) have postulated that there is an EA-sensitive sodium transport system at the serosal side of the renal tubules which is adjacent to the ouabain-sensitive ATPase pump. However, no experimental data were provided to substantiate that the EA-sensitive Na transport system has to be at the serosal side, either in the renal tubule or in the intestinal mucosa. Our experimental data show that when EA was added to the serosal bathing medium at concentrations of 0.1–0.5 m*M*, little effect on PD and I_{sc} measurement was observed. Only when the compound was added to both sides of the intestinal membrane, did EA exhibit a greater inhibition of J_{ms} flux of Na ion than the J_{sm} flux and also inhibit both glucose and L-tyrosine transport against concentration gradients across the intestinal sacs. These results, therefore, suggest that EA probably acts on the brush border side of the intestine rather than on the serosal side. It has been proposed and well recognized that in the intestine there is a Na-glucose cotransport system located at the brush border side in which the Na flux and glucose flux (or amino acid flux) mutually drive each other across the brush border membrane (17). Since in the absence of glucose or in the presence of phlorizin the intestine still can transport Na^+ across the mucosal side albeit a reduced rate, one has to assume that the Na ion enters from the mucosal fluid into the mucosal cell by two separate pathways as has been suggested by Rose and Schultz (18) and Armstrong (19): first, a simple diffusion process which is related to the conductance of the membrane; and second, the cotransporting Na-glucose facilitating diffusion process which is probably electrogenic in nature. EA probably inhibits mainly the cotransporting Na-glucose system and therefore greatly reduces the J_{ms} flux of Na ion when glucose is present in the bathing solution and also reduces the glucose and L-tyrosine transport across the mucosal membrane. However, when the bathing solution contains no glucose, the electrogenic cotransporting Na-glucose diffusing process is not in operation and EA exerts the least effect on Na flux which suggests that this diuretic has the least effect on the simple diffusing process either of Na^+ entry or of Cl^- exit.

Considering the inhibitory effect of EA on Cl ion flux, our data support the findings of Field and his co-workers (20). In an early report, Field (11) demonstrated that the Cl^- transport across the rabbit ileum is mediated through the cyclic AMP system. He and his co-workers subsequently showed that EA at 1 m*M* concentration can reduce the Na and Cl ion transport and that such inhibition correlates with the reduction of cyclic AMP in the intestinal membrane (20).

Contrary to the EA effect, furosemide and amiloride did slightly inhibit the PD and I_{sc} across the mice intestine. Such inhibitory effect on Na ion flux was mainly on the J_{ms}, rather than on the J_{sm} value of Na ion, indicating that the effect of these two diuretics was mainly at the brush-border side of the mucosa. Our data also show that these two diuretics exhibited no inhibitory effect on either glucose or L-tyrosine transport, even at a concentration of 1 m*M*, suggesting that these compounds do not act on the Na-glucose cotransporting process, but proba-

bly act on the simple diffusion process by decreasing the conductance of the membrane to both Na and Cl ions. When the bathing medium contained no glucose as substrate, the net Na ion flux was normally very low in the mice intestine. Statistical analysis shows there is no significance between J_{ms} and J_{sm} values of Na flux. The inhibitory effect of these two diuretics was more pronounced in the Cl flux than in the Na flux. Studies on toad bladder (21) and frog skin (22) have shown that amiloride decreased Na flux across these membranes by restriction of Na entry in the mucosal side, partially by diminishing Na conductance but with no effect on the Na pump in the serosal side. Wolf and Fuelgraff (23) also interpreted the inhibitory effect of furosemide on Na flux as influencing the cell membrane permeability: namely, a change in conductance of the membrane. Kokko (9) and Burg *et al.* (10) have also demonstrated with isolated renal tubule perfusion studies that furosemide acts mainly on the Cl^- transport system at the ascending limb of Henle's loop of the kidney. Such results are similar to what we have found in the intestine.

We have made preliminary studies on the intracellular PD measurement of mouse jejunum (unpublished data) and found that EA causes hyperpolarization of the mucosal-to-cell PD in glucose containing bathing medium but exerts no effect on the intracellular PD in glucose-free medium. However, furosemide and amiloride did produce a hyperpolarization of the intracellular PD in either glucose-free or glucose-present bathing medium. Such results further substantiate our conclusion that EA acts mainly on a Na-glucose cotransport facilitated system; furosemide and amiloride mainly affect the simple ion entry (e.g., Na^+) or ion exit (e.g., Cl^-) process across the brush-border membrane of the intestine.

Summary. The jejunal mucosal membrane of albino mice was used to study the electrical properties and ion transport. The membrane was bathed in Krebs-Ringer solution with or without glucose. When ethacrynic acid (EA), furosemide, or amiloride was added to the bathing fluid of both sides, a transient increase followed by a decrease of both potential difference (PD) and short circuit current (I_{sc}) were observed. In glucose-containing bathing medium, EA inhibited both net Na and Cl flux and residual flux; however, EA had little effect on both Na and Cl flux in glucose-free bathing medium. Studies using everted intestinal sac technique showed that EA inhibited both glucose and L-tyrosine across the mucosal membrane against concentration gradients.

Furosemide and amiloride were less potent than EA in inhibiting the Na and Cl flux when the bathing solution contained glucose. But these two compounds had no effect on glucose and L-tyrosine transport across the intestinal mucosa. Furthermore, they did inhibit Cl flux even in the condition of glucose-free bathing medium. It is postulated that all three diuretics act on the brush-border membrane of the intestine. EA probably inhibits the Na-glucose cotransporting system; furosemide and amiloride inhibit the simple diffusion process of Na entry or Cl exit by decreasing the conductance of the membrane.

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