Laboratory experience for understanding the physiological basis of diuretic therapy

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SCHWAB, THOMAS R., AND FRANKLYN G. KNOX. *Laboratory experience for understanding the physiological basis of diuretic therapy.* Am. J. Physiol. 260 (Adv. Physiol. Educ. 5): S10–S13, 1991.—A combined lecture and laboratory experience was designed to enhance medical school students' understanding of the physiological basis of diuretic therapy. Studies are performed by students in anesthetized dogs to determine the effects of four clinically useful diuretics on renal function. The objective of the experience is to *1*) learn the mechanisms of action, clinical indications, and adverse effects of diuretics; *2*) review the renal physiology of glomerular filtration and sodium metabolism; and *3*) complete analysis and interpretation of experimental data. These sessions provide an effective practical educational experience in applying the scientific method to begin to understand the physiology and pharmacology of diuretics.

physiology education; pharmacology education; diuretics; renal function; student laboratory

DIURETICS ARE AMONG the most commonly prescribed medications worldwide. Hypertension (6), nephrotic syndrome (3), hypercalciuric renal lithiases (11), diabetes insipidus (2), cerebral edema (9), and glaucoma (1) represent the diverse conditions in which diuretic therapy is useful.

The second-year Mayo Medical School pharmacology curriculum has traditionally included one lecture encompassing the pharmacology of diuretics. Before the lecture each student is given a background reading assignment, "Sites of Mechanisms of Action in the Kidney and Effect on Monovalent Ion Excretion" in *The Physiological Basis of Diuretic Therapy in Clinical Medicine* (4). During the lecture the mechanisms of action, clinical uses, and adverse effects of each class of diuretic are sequentially presented in an order based on the principal site of action of the diuretic from the distal to the proximal nephron by a renal physiologist (F. G. Knox) and a nephrologist (T. R. Schwab).

We have recently introduced a new laboratory experience that simultaneously accompanies this traditional lecture format to enhance medical student learning of the physiological basis of diuretic therapy. Our rationale for providing this added visual demonstration of pharmacology is based on the premise that the students' anticipation and excitement for experimentation improves their learning of clinically relevant subject matter. It also provides an important opportunity for the discussion of the use of animals in research. Our objectives for the students include *1*) learning the mechanisms of action, clinical indications, and adverse effects of diuretics; *2*) reviewing the renal physiology of glomerular filtration and sodium metabolism; and *3*) completing experimental data analysis and interpretation using the scientific method.

The specific aim of this laboratory experience is to determine the effects of four clinically useful diuretics on renal function in anesthetized dogs. Previous studies have suggested that renal diuretic and natriuretic responses are blunted during extracellular volume depletion (8). Therefore the present study was performed in the presence and absence of replacement of urinary volume and tested the hypothesis that volume replacement increases the renal effects of the distal nephron diuretic chlorthiazide, the ascending loop of Henle diuretic furosemide, the proximal nephron diuretic acetazolamide, and the proximal and distal nephron diuretic mannitol.

METHODS

Overview. Students were assembled in the laboratory and divided into one of four preassigned diuretic groups and given a short orientation to this five-period protocol, including its purpose and their responsibilities. This was followed by the beginning of the didactic lecture and the protocol's first period. During the first period (placebo), we presented the physiological effects, clinical indications, and adverse effects of dietary sodium restriction and pharmacological aldosterone inhibition. During subsequent periods, we presented similar discussions for each diuretic (chlorthiazide, furosemide, acetazolamide, and mannitol) after its administration by the students. The effects of each diuretic on glomerular filtration rate, proximal and total nephron sodium reabsorption, and potassium and water excretion were determined. Students were asked to consider drug distribution, metabolism, and possible drug-drug interactions in interpreting the data that tested the hypothesis. Each student provided us with an anonymous response regarding the educational value of these 3-h sessions.

Student assignments. Each second-year medical student was assigned to one of two groups of 20 students 1 wk before each session. Two separate 3-h sessions were held. In *student group I*, urinary losses during the protocol (see below) were intravenously replaced by equal

volumes of 0.45% NaCl, and in *student group II*, urinary losses were not replaced. Within each group, students were preassigned to administer one of the four diuretics and received a printed handout including *1*) the goals, aims, and hypothesis of the study; *2*) a copy of the protocol, which was approved by the Institutional Animal Care and Use Committee; *3*) a specific diuretic assignment; *4*) a spreadsheet for data collection and calculations (Table 1); *5*) a listing of useful formulas for data interpretation (Table 2); and *6*) the required reading assignment (4).

Laboratory preparation. During the night before each session, one adult mongrel dog was fasted with free access to water after receiving a 300-mg lithium carbonate capsule by mouth. Lithium clearance was used as an index of sodium reabsorption by the proximal tubule (7). Two hours before each session the dog was weighed and anesthetized with an intravenous injection of pentobarbital sodium followed by intubation of the trachea. A femoral artery was cannulated with a polyethylene catheter for blood sampling and blood pressure monitoring, and a femoral vein was cannulated with two catheters for infusions and diuretic administration. A midline suprapubic incision was made to expose both ureters, which were cannulated for urine collection. The catheters were brought through the incision, and the incision was sutured closed.

After surgical preparation, arterial blood pressure was monitored continuously. For measurement of glomerular filtration rate, an infusion of inulin dissolved in 0.9% NaCl at 1 ml/min to achieve a plasma concentration of 50 mg/dl was started. The animal was allowed to equilibrate for at least 1 h and was given supplemental pentobarbital anesthesia as indicated throughout the study. Intravenous infusions of 0.45% NaCl (*student group I* only) and the following diuretics were prepared for bolus intravenous administration: 100 mg chlorthiazide, 100 mg furosemide, 400 mg acetazolamide, and 10 g mannitol. Each animal was prepared to this point by support personnel.

Protocol. Placebo, chlorthiazide, furosemide, acetazolamide, and mannitol were sequentially administered intravenously over 1 min by the students. After allowing 5 min for distribution of the agent, a 15-min urine collection was completed. An arterial blood sample was collected 7.5 min after beginning each urine collection. In *student group I*, urinary losses were replaced by infusing 0.45% NaCl at a rate adjusted to equal urinary flow rate. Students recorded mean arterial blood pressures and urine volume collected for each period.

Data analysis. At the end of each experiment support personnel killed each dog by intravenous injections of concentrated potassium chloride, and urine and blood samples were analyzed for osmolality and concentrations of sodium, lithium, potassium, and inulin. Each student group received copies of the results from both student groups. Students calculated respective period urinary flow rate, glomerular filtration rate, urinary sodium excretion, fractional excretion of sodium, fractional excretion of lithium, urinary potassium excretion, fractional potassium excretion, and free water clearance.

RESULTS AND DISCUSSION

Results from the sessions are depicted in Table 3 and Figs. 1 and 2. We developed a set of study questions for the students that assisted in the following interpretation of the data.

1. Did the data support the hypothesis that the renal effects of diuretics are increased with volume replacement? Urinary flow rate and sodium and potassium excretion progressively increased in both studies as distal nephron, ascending loop, and proximal nephron diuretics were sequentially administered. The data support the hypothesis that the renal effects were greatest during volume replacement.

2. What effects were seen on the glomerular filtration rate and why? Glomerular filtration rate decreased progressively in each study. Two explanations are possible. Glomerular filtration rate may have decreased due to

TABLE 1. *Data and calculations spreadsheet*

Diuretic	Data Collection										
	15-min urine vol	P_{osm}, mmol/kgH_2O	S_{Na}, meq/l	S_K, meq/l	S_{Li}, meq/l	S_{In}, mg/dl	U_{osm}, mmol/kgH_2O	U_{Na}, meq/l	U_K, meq/l	U_{Li}, meq/l	U_{In}, mg/dl
Placebo											
Chlorthiazide											
Furosemide											
Acetazolamide											
Mannitol											

Diuretic	Calculations								
	$\dot{V}$, ml/min	C_{In}, ml/min	C_{H_2O}, ml/min	$U_{Na}V$, μeq/min	FE_{Na}, %	U_KV, μeq/min	FE_K, %	$U_{Li}V$, μeq/min	FE_{Li}, %
Placebo									
Chlorthiazide									
Furosemide									
Acetazolamide									
Mannitol									

P_{osm}, plasma osmolality; S_{Na}, S_K, S_{Li}, S_{In}, serum Na, K, Li, and inulin concentrations; U_{osm}, urine osmolality; U_{Na}, U_K, U_{Li}, U_{In}, urine Na, K, Li, and inulin concentrations; $\dot{V}$, urinary flow rate; C_{In}, C_{H_2O}, clearance of inulin and free water; $U_{Na}V$, U_KV, $U_{Li}V$, urinary excretion of Na, K, and Li; FE_{Na}, FE_K, FE_{Li}, fractional excretion of Na, K, and Li.

TABLE 2. *Formulas*

	Units	Formula
Urinary flow rate	ml/min	$\dot{V}$
Glomerular filtration rate	ml/min	$(U_{In} \times \dot{V})/P_{In}$
Urinary excretion rate of substance x	μeq/min	$U_x \times \dot{V}$
Fractional excretion of substance x	%	$[(U_x/P_x)/(U_{In}/P_{In})] \times 100$
Free water clearance	ml/min	$\dot{V} \times [1 - (U_{osm}/P_{osm})]$

$\dot{V}$, urinary flow rate; U_{In}, urine inulin concentration; P_{In}, plasma inulin concentration; U_x, urine concentration of substance x; P_x, plasma concentration of substance x; U_{osm}, urine osmolality; P_{osm}, plasma osmolality.

TABLE 3. *Results before and after diuretic administration*

	$\dot{V}$, ml/min	C_{In}, ml/min	C_{H_2O}, ml/min	$U_{Na}V$, μeq/min	FE_{Na}, %	U_KV, μeq/min	FE_K, %	$U_{Li}V$, μeq/min	FE_{Li}, %
No volume replacement									
Control	1.1	89	−1.4	184.4	1.4	70.8	24.2	14.3	40.3
Chlorthiazide	2.7	76	−1.0	411.5	3.7	104.0	44.2	5.3	17.5
Furosemide	5.5	54	−0.4	699.4	8.8	143.9	83.4	5.5	26.8
Acetazolamide	9.3	50	−0.2	1,185.3	16.2	226.8	151.5	7.5	68.3
Mannitol	10.0	34	0.4	1,125.0	23.1	187.0	199.3	6.5	86.9
Volume replacement									
Control	0.2	74	−0.9	69.1	0.6	14.0	5.0	1.9	25.2
Chlorthiazide	3.1	61	−2.0	519.5	5.8	137.2	58.8	2.9	47.2
Furosemide	10.8	61	−0.8	1,483.9	16.8	191.2	89.6	3.6	58.7
Acetazolamide	12.2	57	−0.8	1,660.4	19.9	229.4	119.9	3.5	68.0
Mannitol	14.0	56	−2.0	1,689.8	21.6	190.4	136.8	4.0	90.6

See legend to Table 1 for definitions of abbreviations.

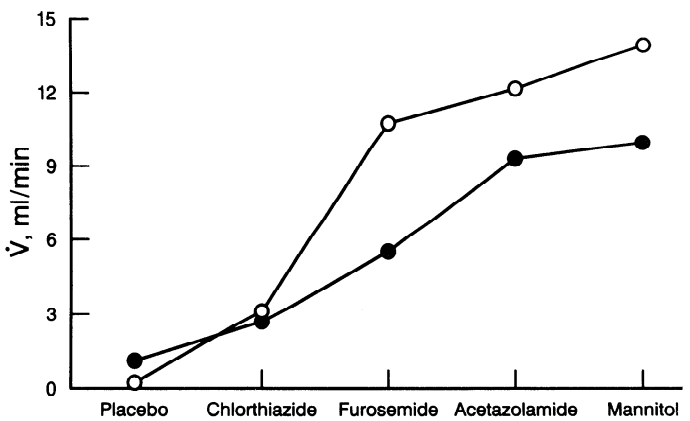

FIG. 1. Urinary flow rate ($\dot{V}$, ml/min) before and after diuretic administration in presence (open circles) and absence (solid circles) of replacement of urinary volume losses with equal volumes of 0.45% NaCl.

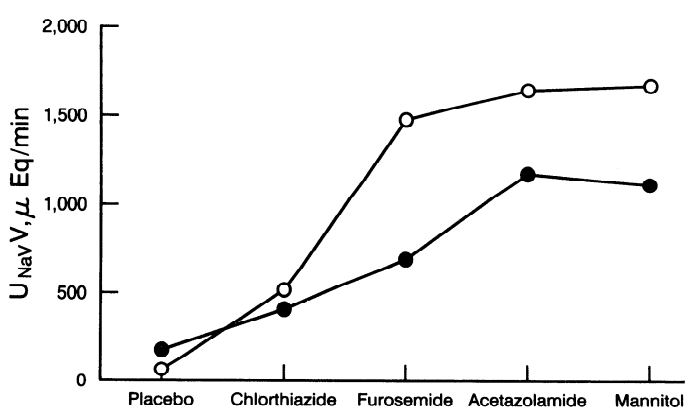

FIG. 2. Urinary sodium excretion ($U_{Na}V$, μeq/min) before and after diuretic administration in presence (open circles) and absence (solid circles) of replacement of urinary volume losses with equal volumes of 0.45% NaCl.

intrarenal hemodynamic compensation for extracellular volume depletion, and the increased diuretic-induced distal nephron sodium delivery may have stimulated tubuloglomerular feedback.

3. What is the significance of the observed changes in the fractional excretions of sodium, lithium, and potassium? The total filtered load of any substance can be determined once the glomerular filtration rate is measured. The fractional excretion of a substance is the percentage of the total filtered load of that substance in the final urine. It reflects the net tubular handling of a substance whether reabsorption, secretion, or both.

The fractional excretion of sodium increased throughout both studies, demonstrating progressive decreases in tubular reabsorption of sodium.

The fractional excretion of lithium, an index of proximal tubular sodium reabsorption, decreased in the absence of volume replacement during chlorthiazide and furosemide administration. This is consistent with previous studies in which proximal tubule sodium reabsorption increases as compensation for the pharmacologically induced distal nephron natriuresis (5).

The fractional excretion of potassium increased throughout both studies and eventually exceeded 100%, demonstrating net renal tubular potassium secretion occurred.

4. How was the clearance of free water affected during the experiment? The clearance of free water was negative at baseline and remained negative thoughout the studies. The most likely explanation for this finding is that the thiazide diuretic persistently inhibited the diluting segment of the nephron and interfered with the renal production of "free water" (10).

5. What limitations can be identified in this investigation's methods? Only one experiment was conducted in each group, and it is likely that at least an additional

five studies are necessary in each to define statistical significance and biologic variability.

The effects of each diuretic were likely cumulative given the short protocol and the half-life of each agent. Data from periods after administration of the first diuretic may be additive and should not be attributed solely to the subsequent diuretic. Drug-drug interactions and relative diuretic dose relationships may also play a role in the results.

SUMMARY

These laboratory sessions provide an effective practical experience in applying the scientific method to begin to understand the physiology and pharmacology of diuretics. It stimulates students to review renal physiology and to calculated and interpret measurement of several renal functions from a study they perform. Of the students responding to our questionnaire, 72% felt this laboratory was a good to excellent learning experience.

In conclusion, we believe these sessions, which combine didactic discussions and a laboratory experience, not only enhance learning of the physiological basis of diuretic therapy but also aid in development of an appreciation for the contributions of laboratory investigations to clinical medicine.

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