Effect of Ouabain on Na^+ and K^+ Excretion in the Rat.* (32428)

A. G. RAMSAY AND G. SACHS (Introduced by H. C. Elliott)
Departments of Medicine, and Physiology and Biophysis, University of Alabama, Birmingham

Ouabain at concentrations ranging from 10^{-3} to 10^{-7} inhibits Na^+ transport and transport ATPase in a number of tissues(1). In the kidney, ouabain inhibits renal tubular reabsorption of sodium in several species(2,3). Such an effect on the proximal tubule would increase the delivery of sodium to the distal segment. Additionally, inhibition of distal Na^+ transport by ouabain, as has been shown to occur in the K^+ loaded chicken(3), would diminish K^+ excretion by reduction of Na^+ — K^+ exchange. On the other hand, if ouabain has little or no distal effect, augmentation of cationic exchange and increased K^+ excretion would occur as a result of the Na^+ load. Accordingly, clearance experiments were performed in order to determine which of these two mechanisms occurs in the rat.

Materials and methods. Clearance experiments were performed on the left kidney of 50 white male rats. The animals were anesthetized with intraperitoneal sodium ethyl-(1-methylpropyl)-thiobarbiturate in dosages of 70 mg/kg of body weight. The trachea was cannulated. The left kidney and ureter were exposed through an abdominal incision and the ureter was cannulated with #10 P.E. tubing. Periodic aspirations of the bladder by needle were made throughout the experiments. NaCl 0.85%, mannitol 5% and inulin 0.75% were infused at 1.2 ml/hr into the left jugular vein of 43 rats by a Harvard infusion pump. An inulin prime of 20 mg in saline was given at the start of infusion. The left renal artery was infused with 0.85% NaCl at 300 μl/hr through a 30 gauge needle connected to a second infusion pump by #10 P.E. tubing. After a 30 minute period of equilibration, four 60 minute collections of urine were made under mineral oil. After the first clearance period which served as control, the renal artery infusion was changed to ouabain in saline in concentrations varying from 1.37×10^{-4} M to 1.37×10^{-2} M in 33 rats. Renal arterial infusion of saline was continued throughout the experiment in 10 animals. These rats served as further controls. In order to compare the effects of ouabain with saline diuresis, 7 rats were infused systemically with 5% NaCl, 5% mannitol and 0.75% inulin at 1.2 ml/hr. No renal arterial infusion was administered to these animals, but otherwise the experimental protocol was the same. At the conclusion of experiments, 6 ml of heparinized blood was taken from the aorta.

Inulin in plasma and urine was determined by the method of Schreiner(4), and sodium and potassium by flame photometry. pH of urine was determined with a Radiometer pH meter. GFR was taken as the clearance of inulin.

Results. Renal arterial infusion of ouabain produced a significant increase in urinary excretion of potassium in all experiments. However, it produced Na^+ diuresis in only 18 of 33 experiments. Consequently, data were subdivided into 2 groups, first those animals with sodium diuresis and second, those experiments which did not show this effect. The absence of Na^+ diuresis in the second group could not be accounted for by the concentration of infused ouabain. This group contained animals infused with both the high and low concentrations. Similarly, Na^+ diuresis in the first group occurred with both concentrations of ouabain. Table I summarizes data from the group with Na^+ diuresis. Renal arterial infusion of ouabain produced more than a 3-fold increment in K^+ excretion from 493 to 1825 nM/min. Sodium excretion was increased from 212 to 868 nM/min and there was more than a 2-fold increase in rate of urine flow. There was little appreciable change in GFR. The data for Na^+ and K^+ excretion and GFR in this same group are graphically depicted by the solid points and lines in Fig. 1. The open circles joined by broken lines show data obtained from the 10 animals in which saline was infused into the renal artery

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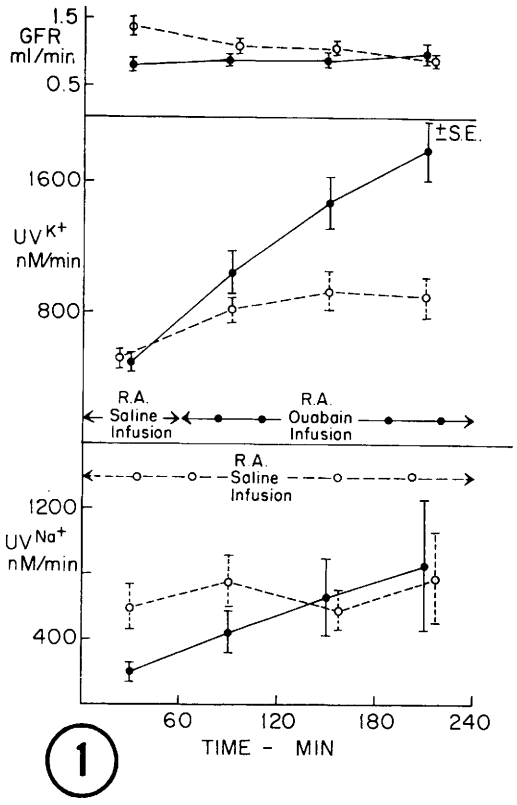
TABLE I. Effect of Ouabain on K^+ (UV^{K+}) and Na^+ (UV^{Na+}) Excretion, Urine Flow (V) and GFR in 18 Animals with Na^+ Diuresis. Mean values ± SEM.

	Control	Ouabain 1	Ouabain 2	Ouabain 3
UV^{K+}, nM/min	493 ± 65	1046 ± 145	1470 ± 155	1825 ± 207
UV^{Na+}, nM/min	212 ± 39	441 ± 142	665 ± 256	868 ± 423
V, μl/min	7.87 ± .59	11.28 ± 1.07	13.73 ± 1.26	16.94 ± 1.64
GFR, ml/min	.79 ± .09	.92 ± .07	.97 ± .10	1.05 ± .18



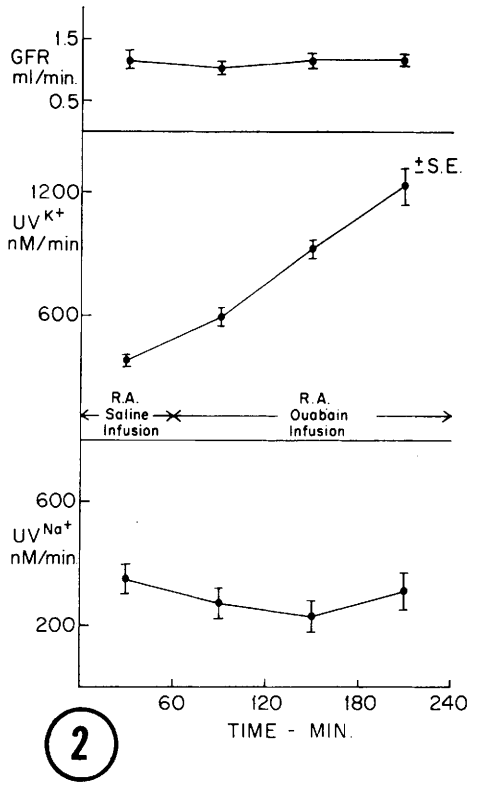


FIG. 1. Effect of ouabain infusion (solid points and lines) and saline infusion (open points and broken lines) into renal artery on K^+ (UV^{K+}) and Na^+ (UV^{Na+}) excretion and GFR. Mean values ±SEM. Results in 18 animals in which Na diuresis occurred.

FIG. 2. Effect of renal artery infusion of ouabain on K^+ (UV^{K+}) and Na^+ (UV^{Na+}) excretion, and GFR. Mean values ± SEM. Results in 15 animals in which Na^+ diuresis did not occur.

throughout the course of the experiment. The striking increase in both Na^+ and K^+ excretion in the ouabain infused kidneys compared to the saline infused kidneys is quite apparent. Na^+ excretion in the saline infused kidneys was initially greater than in the ouabain experiments. However, there was no appreciable increase of UV^{Na+} with time in the control group.

Table II is a summary of the data from 15 rats in which renal arterial infusion of ouabain did not increase the rate of Na^+ excretion. Actually there was a slight decrease from 347 to 313 nM/min. This occurred in the absence of any appreciable effect on GFR. However, there was a marked increase in UV^{K+} from 395 to 1239 nM/min. This was associated with a small increment in the rate of urine flow. The data from this group are graphically depicted in Fig. 2.

TABLE II. Effect of Ouabain on K^+ and Na^+ Excretion, Urine Flow and GFR in 15 Animals Without Na^+ Diuresis. Mean ± SEM.

	Control	Ouabain 1	Ouabain 2	Ouabain 3
UV^{K^+}, nM/min	395 ± 32	597 ± 42	930 ± 53	1239 ± 86
UV^{Na^+}, nM/min	347 ± 56	273 ± 50	227 ± 51	313 ± 63
V, μl/min	8.20 ± .48	8.45 ± .48	9.73 ± .60	10.80 ± .94
GFR, ml/min	1.16 ± .10	1.02 ± .04	1.16 ± .08	1.16 ± .05

In all experiments, ouabain produced a significant increase in acidification of the urine. Urine H^+ concentration increased from 1.2 to 2.8 μM/l (Fig. 3).

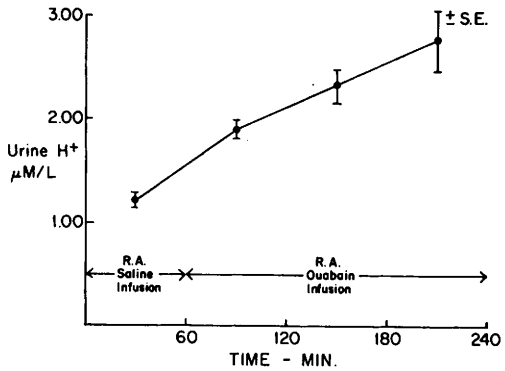


FIG. 3. Progressive acidification of urine by renal artery infusion of ouabain.

It is generally agreed that the bulk of K^+ excreted in the urine is the result of secretion of this ion by the distal tubule(5). Potassium secretion is probably the result of two factors, the magnitude of the potential difference and Na^+ — K^+ exchange in the distal tubule. Infusion of hypertonic saline has been shown to augment distal tubular secretion of K^+ by increasing the potential difference(6). As hypertonic saline actually decreases distal tubular Na^+ reabsorption (6), it is unlikely that Na^+ — K^+ exchange plays a significant role in the kaluresis of saline infusion. It should be a reasonable assumption then, that for any given rate of Na^+ excretion, K^+ excretion should be greater when both the cation exchange and electrical gradient mechanisms are operative than when K^+ secretion depends almost entirely on the magnitude of the potential difference. Consequently, a comparison was made between the Na^+ and K^+ excretion in 7 animals infused with 5% NaCl and the excretion of these ions in the 18 animals in which ouabain produced Na^+ diuresis (Fig. 4). The solid

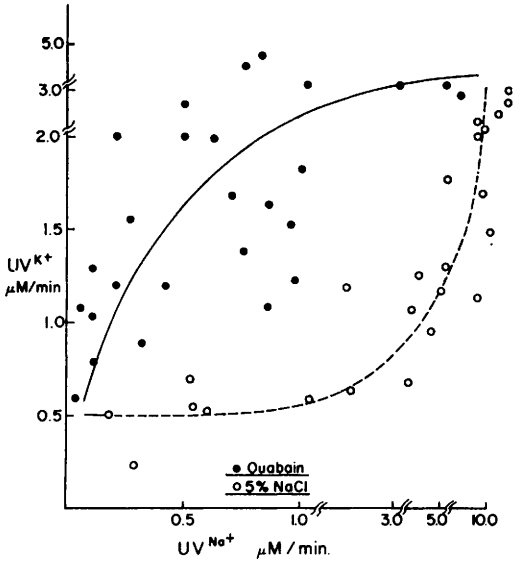


FIG. 4. Comparison of rates of excretion of Na^+ and K^+ with ouabain (solid points) and hypertonic saline (open points). Lines through respective points plotted by eye.

points show data from ouabain infused animals and the open circles show data from animals infused with hypertonic saline. It can be seen that for any given rate of Na^+ excretion, ouabain produced a considerably greater rate of K^+ excretion than did hypertonic saline.

Discussion. From the results described above, it would appear that there is a distinct species difference between the effect of ouabain on the renal tubule of dog and chicken on one hand(2,3), and on the renal tubule of rat on the other. In the dog, ouabain uniformly increases the rate of Na^+ excretion, reduces H^+ secretion and has little effect on K^+ excretion. Similar results have been shown to occur in the chicken, and after loading with potassium sulphate ouabain significantly re-

duced K^+ excretion(3). In the rat, ouabain uniformly increased both K^+ and H^+ excretion, but Na^+ diuresis was not a consistent finding and occurred in only half of experiments.

In the proximal tubule of Necturus, it has been shown that ouabain acts directly on the renal tubular cell to inhibit the reabsorption of Na^+ from tubular fluid(7). The effect occurs with ouabain on either the luminal or contraluminal side of the tubule. Unfortunately, there is no direct micropuncture study of the effect of ouabain in the distal tubule. The results of clearance studies in dog and chicken indicate that ouabain inhibits Na^+ reabsorption distally(2,3). The mechanism by which ouabain influences tubular transport of sodium is obscure. Presumably it is the result of inhibition of a transport ATPase as has been shown to occur in a variety of other transporting tissues(1).

In toad bladder ouabain has been shown to have a biphasic effect(8). In low concentration it accelerates Na^+ transport, whereas in high concentration Na^+ transport is inhibited. Without accurate measurements of renal blood flow, it is impossible to estimate the concentration of ouabain which perfused the kidney in our experiments. However, it is unlikely that the absence of Na^+ diuresis in one half the experiments was dose related. This was observed with renal artery infusions of 1.37×10^{-4} M and infusions of 1.37×10^{-2} M. Furthermore the increment in K^+ excretion was as great with the lower ouabain concentration as it was with the higher concentration.

Two mechanisms by which ouabain could influence the renal tubular transport of Na^+, K^+ and H^+ in the rat must be considered. The most likely possibility is that ouabain has a biphasic action on tubular transport whereby Na^+ reabsorption is inhibited proximally but is stimulated distally. Inhibition of proximal tubular reabsorption of Na^+ would augment the delivery of Na^+ to the distal tubule. Enhancement of Na^+ reabsorption at this site by the action of ouabain would increase Na^+ — K^+ and Na^+ — H^+ exchange. This could account for the consistent increase in the excretion of both H^+ and K^+ with renal artery infusion of ouabain. If stimulation of distal tubular Na^+ reabsorption was of sufficient magnitude, it is conceivable that the increment of Na^+ delivery to the distal tubule produced by inhibition of proximal tubular transport might be entirely reabsorbed. Under these circumstances, sodium diuresis would not occur, such as observed in half of our experiments. On the other hand, if the delivery of Na^+ from the proximal tubule was so great that the augmented capacity of the distal tubule for reabsorption was exceeded, then Na^+ diuresis might be expected such as seen in the remainder of our experiments. The concept of stimulation by ouabain of distal tubular Na^+ reabsorption, and Na^+ — K^+ and Na^+ — H^+ exchange is supported further by the observation that for any given rate of Na^+ excretion, ouabain produced a greater rate of K^+ excretion than did infusion of 5% NaCl (Fig. 4). Hypertonic saline infusion will inhibit Na^+ reabsorption in the proximal tubule, increase the delivery of Na^+ to the distal tubule and create a diuresis of both Na^+ and K^+(6,9). Further, it has been shown that increased secretion of K^+ in saline diuresis is primarily the result of augmentation of distal tubular potential difference, and distal Na^+ reabsorption is actually diminished(6). This should decrease Na^+ — K^+ exchange. When both the cationic exchange mechanisms and distal tubular potential contribute to K^+ secretion, a greater rate of K^+ excretion would be expected for any given rate of Na^+ excretion than when K^+ secretion depends mostly on electrical potential. It is possible that ouabain may act only on the proximal tubule and have no effect distally. Inhibition of proximal tubular Na^+ reabsorption with no change of distal reabsorption could increase distal K^+ secretion by the electrical potential mechanism. However, this postulate for ouabain action in rat tubule seems unlikely as it would be expected that Na^+ diuresis would be produced consistently.

Summary. The effect of renal artery infusion of ouabain on urinary excretion of Na^+, K^+ and H^+ was investigated by clearance methods in the rat. Ouabain consistently produced a 3-fold increase in K^+ excretion

and over a 2-fold increase in urinary H^+ concentration. The effect on Na^+ excretion was variable. About half the animals responded with a 4-fold increase in UV^{Na^+} and 2-fold increment in urine flow. The remainder showed no change in Na^+ excretion. It appears that there is a definite species difference in the response to ouabain of the rat kidney on one hand, and the chicken and dog kidney on the other. The most likely explanation of the phenomenon noted in the rat is that ouabain may have a biphasic effect whereby proximal reabsorption of Na^+ is inhibited and distal reabsorption, including cationic exchange for K^+ and H^+ is stimulated.

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Response of Splenectomized Mice to Bacterial Agents.* (32429)

GARY R. JOHNSON, HAROLD N. CARLISLE, AND SAMUEL SASLAW
Department of Veterinary Pathology, College of Veterinary Medicine and Division of Infectious Diseases, Department of Medicine, College of Medicine, The Ohio State University, Columbus

Previous studies from this laboratory showed that splenectomized and normal monkeys responded similarly after infection with streptococci(1), pneumococci(2) or staphylococci(3). Results of studies on the role of the spleen in resistance to infection employing other experimental animals have varied depending on species, infective agent and route of inoculation employed(4). The present report is concerned with mortality following intravenous, intraperitoneal, intranasal or aerosol challenge of white mice with various bacteria at different intervals after splenectomy.

Materials and methods. White Swiss mice of both sexes, weighing 14-16 g, were splenectomized under ether anesthesia. Non-splenectomized controls were similarly treated, except that the spleen was manipulated, but not removed. *Streptococcus hemolyticus,* Group A (Stollerman strain T14), *Staphylococcus aureus,* Smith strain, *Diplococcus pneumoniae,* type III (ATCC No. 6303), and a *Pseudomonas aeruginosa* isolated from a patient at University Hospital were utilized.

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Streptococcal and staphylococcal inocula consisted of dilutions of 6-hour Trypticase Soy (BBL) broth cultures inoculated from blood agar plates incubated 24 hours. Pneumococci and the pseudomonas were grown for 18 hours on blood agar plates inoculated from 8-hour Todd-Hewitt (Oxoid) and Trypticase Soy broth, respectively. Inocula to be given intraperitoneally, intravenously and intranasally were prepared in physiologic saline. Suspensions of the pneumococcus used in aerosol challenge of mice in a Model 3 Henderson apparatus(5) were prepared in 0.1% Bacto-gelatin (Difco) in physiologic saline.

Results. As seen in Table I, no significant differences in mortality rates were observed among splenectomized, sham-operated and normal mice inoculated intravenously with the streptococcus 4 hours, 1, 3, 7, 14 and 21 days after operation. For example, in mice inoculated with 7.4×10^4 organisms as early as 4 hours after operation, 24 of 30 (80.0%), 21 of 30 (70.0%) and 25 of 30 (83.3%) deaths occurred in splenectomized, sham-operated and normal mice, respectively.