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Received October 21, 1964. P.S.E.B.M., 1965, v118.

Effect of Furosemide on Renal Medullary Sodium Gradient.* (29846)

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Furosemide (4-chloro-N-(furylmethyl)-5-sulfamoylanthranilic acid) has been shown to be an effective diuretic-saluretic agent in both man and laboratory animals(1,2,3). Though similar in chemical structure to the thiazide diuretics(1), furosemide is capable of producing a greater maximal response than hydrochlorothiazide(1). Suki *et al*(4) have reported that furosemide impairs the concentrating ability of the kidney. The process of urinary concentration is dependent upon the renal medullary sodium concentration which increases toward the papilla. This gradient is believed to result from the reabsorption of sodium from the ascending limb of the loop of Henle(5) and Suki *et al*(4) have proposed that furosemide acts in this area of the nephron. An agent that inhibits sodium reabsorption in the ascending limb of the loop of Henle would be expected to abolish the medullary sodium gradient. Thus, the purpose of this investigation was to evaluate the effect of furosemide on the renal medullary sodium gradient in the dog.

Methods. Seven female mongrel dogs weighing 6.5 to 12 kg were used. Food and water were withheld 18 hours prior to the experiments. The animals were anesthetized with pentobarbital sodium (30 mg/kg, intravenously) and tracheotomized. Carotid blood pressure was monitored by means of a Statham arterial pressure transducer and a Gilson direct-writing oscillograph. Isotonic sodium chloride was infused intravenously at a rate of 0.5 ml/kg/minute. The left kidney was exposed by means of a flank incision and the ureter was cannulated. When a quantity of urine sufficient for analysis (2 ml) had been collected, the renal pedicle was ligated and the kidney removed for analysis. Furosemide (25 mg/kg) was then administered intravenously and the saline infusion was replaced with a saline solution containing furosemide (25 mg/kg/hr). The right kidney was then exposed through a flank incision and the ureter was cannulated. Thirty to forty minutes after furosemide infusion was begun, a sample of urine was collected and the kidney was removed. To monitor changes that might occur with time, 4 animals did not receive furosemide but were infused only with saline after removal of the left kidney.

Immediately upon removal of a kidney from an animal, a cross-sectional slice was taken from the organ. From this slice, duplicate samples (50-100 mg) of tissue were obtained from the papillary, middle and basal regions of the medulla and from the cortex.

* Supported in part by U. S. Public Health Service research grants AM-05298 from Nat. Inst. of Arthritis & Metab. Dis. and 1-Fl-GM-25,075 from Nat. Inst. of General Med. Sci.

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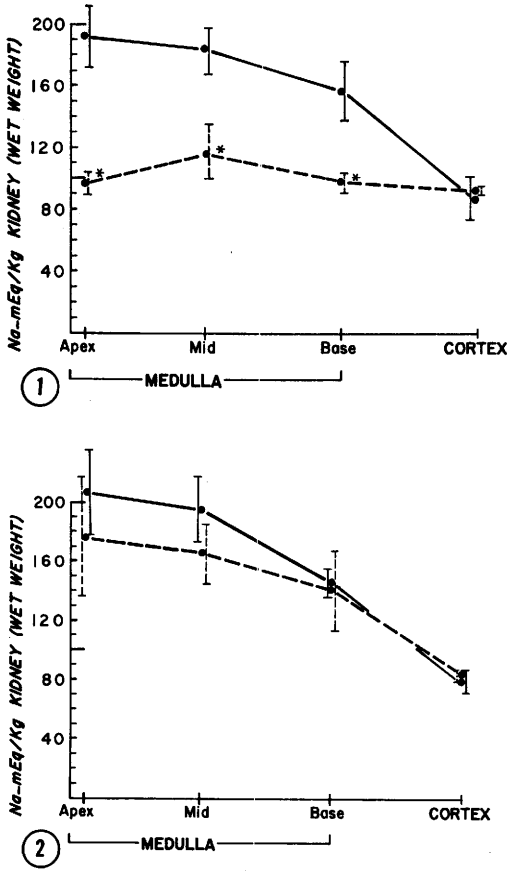

FIG. 1. Effect of furosemide on renal medullary sodium concentration. Solid line represents control values (left kidney) and broken line indicates values after furosemide (right kidney). Vertical lines indicate standard errors. N = 3. * Indicates significant difference ($p < .05$).

FIG. 2. Effect of saline infusion on renal medullary sodium concentration. Solid line represents control values (left kidney) and broken line indicates values after saline infusion (right kidney). Vertical lines represent standard errors. N = 4.

Each sample was placed in a 10 ml volumetric flask and the wet weight of the tissue recorded. From removal of the kidney to completion of this step required no more than 2 minutes. The tissue was then digested with 0.5 ml of concentrated nitric acid and analyzed for sodium using a Coleman flame photometer (Model 21). Values obtained are reported as mEq of sodium per kg of tissue (wet weight). Urinary sodium concentration and osmolarity were also measured, the latter with a Fiske osmometer. Duplicate samples were averaged and these averages were used to compute the reported means (± standard errors). The data were analyzed statistically using the Student "t" test (6). The 0.05 level of probability was used as the criterion of significance.

Results and discussion. Intravenous administration of furosemide (25 mg/kg/hr, 25 mg/kg prime) was found to abolish the medullary sodium gradient (Fig. 1). The sodium content of the medullary tissue was significantly greater than that of cortical tissue in the left (untreated) kidneys. However, no such difference was observed after furosemide. To rule out the possibility that the sodium gradient had been altered by the saline infusion, 4 dogs were infused with saline for a period of time equal to that of the furosemide treated animals. The medullary sodium gradient was not significantly affected by the saline infusion (Fig. 2).

During maximal saluresis produced by hydrochlorothiazide, furosemide has been shown to produce a diuretic-saluretic response (7). This suggested that furosemide acts by a different mechanism or in a different site than hydrochlorothiazide. During maximal saluresis produced by ethacrynic acid, furosemide was without effect(7). This suggested a similarity in the mode or site of action of these two compounds. In experiments similar to those reported here, hydrochlorothiazide did not affect medullary sodium gradient (8). Other findings favor a more distal site of action of hydrochlorothiazide(9,10,11). Ethacrynic acid has been found to impair urinary concentration(12,13) and to abolish the medullary sodium gradient(14). Both of these actions are consistent with an inhibition of sodium reabsorption in the ascending limb of Henle and are similar to the findings with furosemide.

Furosemide significantly depressed urinary sodium concentration and total osmolarity. Sodium concentration was decreased from 256 ± 34 to 115 ± 9 mEq/l and urinary osmolarity decreased from 1006 ± 192 to 331 ± 15 mOsm/l. The saline infusion alone produced no significant alterations. These effects are to be expected if sodium reabsorption in the ascending limb of Henle is blocked.

The findings reported here support the conclusions of Suki *et al*(4) that furosemide acts upon the ascending limb of the loop of Henle. These observations also indicate that furosemide and hydrochlorothiazide act in different sites as has been suggested previously(7) and in addition are in agreement with the previous suggestion that furosemide and ethacrynic acid have a common site of action(7).

Summary. Furosemide, 25 mg/kg/hr (25 mg/kg prime) abolished the sodium medullary gradient in the dog kidney. It is concluded that the natruresis produced by furosemide is due, at least in part, to inhibition of sodium reabsorption in the ascending limb of the loop of Henle.

The authors wish to thank Lloyd Brothers, Inc. for supplying the furosemide used in this study. Technical assistance of Mrs. Penny Lang is gratefully acknowledged.

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Received October 28, 1964. P.S.E.B.M., 1965, v118.

Response of Reticuloendothelial System to Experimental Allergic Orchitis in a Genetically Susceptible and Resistant Mouse Genotype.* (29847)

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Data recently obtained in our laboratory seem to point to a highly important role of the reticuloendothelial system (RES) in the pathogenesis of experimental autoimmune disease. Experiments with 2 mouse strains, BSVS and BRVR, have shown that the former responded to a preparatory injection of pertussis vaccine followed by 2 intracutaneous injections of unpurified mouse brain in Freund's adjuvant with the development of allergic encephalomyelitis while the latter failed to acquire the disease(1). Both strains exhibited a significant rise of their RES activity regardless of whether this was measured with the carbon clearance test of Halpern *et al* or by counting the acid phosphatase positive cells in liver and spleen(2,3). While either strain showed increased activity in comparison with its untreated controls no statistically significant difference was noted when the treated animals of the 2 strains were compared with each other. In the present study this investigation was extended to a second autoimmune disease, allergic orchitis.

Materials and methods. For immunization, the same schedule was used as in the production of allergic encephalomyelitis. BSVS and BRVR mice received 0.5 ml of a 1:5

* These investigations were supported by U.S. P.H.S., Grant E4196 obtained by the author while a member of the Medical Faculty, Free University of Berlin, Germany.