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* Present address: Department of Pharmacy, The University, Nottingham, England.

Renal Effects of a Vasodilator: 2-[*N*-methyl-piperidyl-(4)]-3-amino-5-(4′-pyridyl)-pyrazole HCl (Ciba 31-531 Ba)

RONALD L. WILLIAMS and JAMES E. PEARSON

Abstract □ Acetylcholine (Ach), when infused directly into the renal artery of a kidney, causes unilateral vasodilatation and increased salt excretion. Ciba 31-531 Ba, a nonspecific vasodilator, was infused both directly into the renal artery and given systemically, and the effects were compared to Ach. Infusion of Ach at 0.1 μg./kg./min. into the left renal artery (LRA) of an anesthetized dog effected a unilaterally increased salt excretion and increased effective renal plasma flow (ERPF) and urine volume, but no demonstrable change in glomerular filtration rate (GFR) or blood pressure. Infusion of Ciba 31-531 Ba (LRA) at 0.1 mg./kg./min. resulted in a similar unilateral saluresis of approximately 50% increase over Ach with an increase in ERPF and urine volume but no change in GFR or blood pressure. The right kidney served as an internal control. When Ach and Ciba 31-531 Ba were given systemically, at higher doses there was a drop in blood pressure, decreased solute excretion, and decreased ERPF and GFR for both drugs. The effects of Ach were reversed with atropine. The effects of Ciba 31-531 Ba were not reversed by atropine or propranolol. The authors believe that the direct renal effects of these drugs are the results of direct tubular effects associated in some way with hemodynamic alterations.

Keyphrases □ 2-[*N*-methyl-piperidyl-(4)]-3-amino-5-(4′-pyridyl)-pyrazole HCl (Ciba 31-531 Ba)—renal effects □ Renal infusion—Ciba 31-531 Ba □ Acetylcholine, Ciba 31-531 Ba renal effects—comparison □ IV infusion, Ciba 31-531 Ba—renal effects

A new vasodilator, recently synthesized by Ciba (1), given systemically appeared to have contradictory effects on the kidney (2). In some cases it acted as a vasodilator and increased renal blood flow. In other experiments the various systemic effects appeared to predominate and no change or decrease in renal blood flow was observed.

Ciba compound 31-531 Ba, 2-[*N*-methyl-piperidyl-(4)]-3-amino-5-(4′-pyridyl)-pyrazole HCl, antagonized the effects of epinephrine, norepinephrine, angiotensin II-amide, and histamine on smooth muscle of the guinea pig and the cat. It did not antagonize the effects of $BaCl_2$, acetylcholine, or bradykinin (1).

When compared to papaverine, nitroglycerin, and isoproterenol in an open-chest cat preparation where it is possible to measure both systemic arterial and venous pressure, isoproterenol and Ciba 31-531 Ba administration caused both arterial and venous pressure to fall.

The direct renal effects of adrenergic and cholinergic agents have recently been reported in the literature (3). It was felt that a study of this rather nonspecific muscle-relaxing drug on renal function as compared to acetylcholine would be of interest. The following experiments were designed to study the effect on sodium excretion and renal hemodynamics when Ciba 31-531 Ba is given directly into the renal artery and when given systemically.

MATERIALS AND METHODS

Eight mongrel dogs were anesthetized with intravenous injections of pentobarbital sodium, 30 mg./kg. Both ureters were cannulated through an abdominal midline incision, and the cannulae were positioned approximately 1.27 cm. (0.5 in.) below the ureteral pelvic junction. A femoral vein and artery were cannulated and the arterial cannula was connected to an E & M linear transducer (E & M Co., Inc.) with a three-way stopcock for recording blood pressure with an E & M polygraph. Arterial blood samples were obtained through the three-way stopcock. The sustaining solution containing 1.8 mg./ml. of creatinine, 0.5 mg./ml. of *p*-aminohippurate (PAH) in normal saline was infused at a rate of 5 ml./min. through the venous system by means of a dual-syringe constant-flow pump. After exposing the left renal artery by the retroperitoneal approach, a 27-gauge hypodermic needle, attached to No. 10 polyethylene tubing, was placed into the left renal artery in the direction of blood flow. Through this renal arterial system, a solution of isotonic sodium chloride was continuously infused at a rate of 0.1 ml./min. Solutions of drugs were also infused at the same rate through the system by changing the renal arterial infusate to one containing test drugs dissolved in normal saline. One to two hours were allowed for equilibration and then collections of 10-min. urine samples from each kidney were begun. Blood samples, drawn every 20 min., were heparinized, centrifuged, and the plasma immediately removed. At

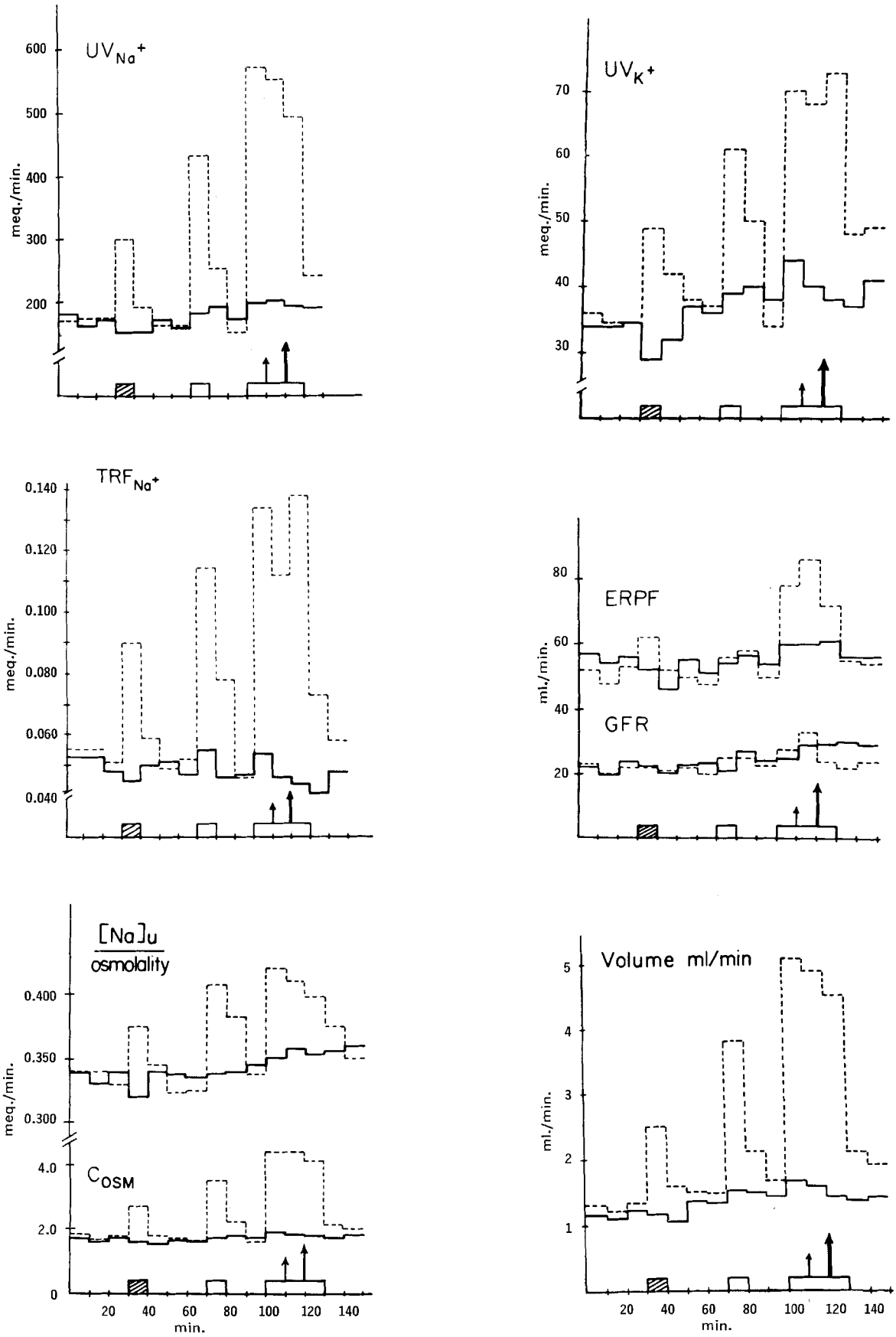


Figure 1.—*Comparison of the direct renal effects of Ciba 31-531 Ba and acetylcholine during infusion into the left renal artery of a 15.5-kg. female dog. Note that the infusion dosage of Ciba 31-531 Ba is 1000 times greater than acetylcholine. Key: experimental (left kidney), - - -; control (right kidney) ——; ▨ acetylcholine (0.1 mcg./kg./min.); □, Ciba 31-531 Ba (0.1 mg./kg./min.); →, atropine (0.1 mg./kg. i.v.); →, propanolol (0.33 mg./kg. i.v.).*

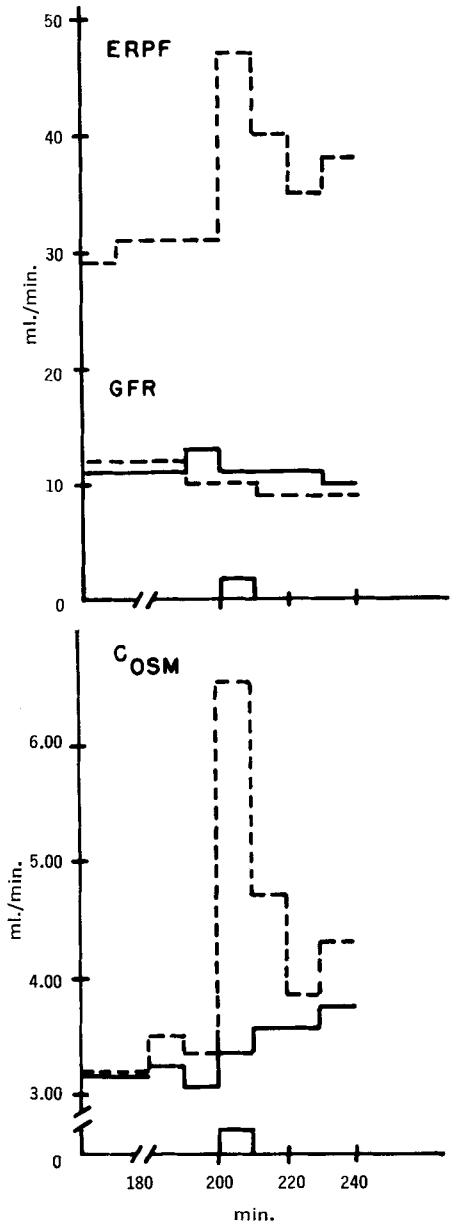
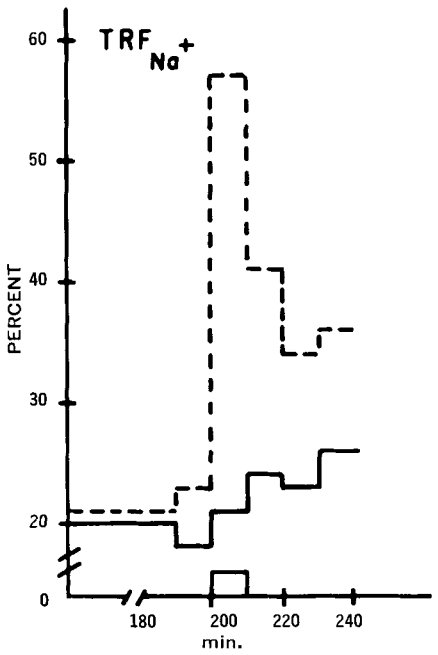


Figure 2—*Effect of Ciba 31-531 Ba during infusion into the left renal artery of a dog with oliguria which had to be given 500 ml. of a solution of 6% mannitol and 2% sodium chloride. The PAH clearances on the right kidney were not determined. Key: experimental (left kidney), - - -; control (right kidney), ——; Ciba 31-531 Ba (0.1 mg./kg./min.), □.*



least three control urine samples were collected before test agents were infused into the kidney. Intravenous experiments used the same technique with sham retroperitoneal exposure.

ANALYTICAL

Chloride concentrations in urine and plasma were determined using a Buchler-Cotlove chloridometer, sodium and potassium concentrations in urine and plasma were determined using a Baird-Atomic flame photometer with an internal lithium standard, a modification of the Bonsnes and Taussky (4) method was used in the determination of plasma and urine creatinine, and PAH determinations were made using a modification of the method of Bratton and Marshall (5). The osmolalities were determined with a Fiske osmometer. In addition to the electrolyte excretion rates, the glomerular filtration rates (GFR), the effective renal plasma flow (ERPF), and the tubular rejection fraction (TRF) of sodium were calculated. The tubular rejection fraction is the ratio of the rate of sodium excretion to the filtered load of sodium. The TRF can vary independently of changes in filtered load (6). Because only concentrations are used in the calculation of TRF, its magnitude is independent of the size of the animal and facilitates comparisons between dogs. The absolute rate of tubular reabsorption (T_{Na}) is less useful because it is markedly affected by filtered load, which in turn is a product of extrarenal as well as renal factors:

$$TRF_{Na} = \frac{U_{Na}V}{P_{Na}GFR} = \frac{U_{Na}P \text{ creatinine}}{P_{Na}U \text{ creatinine}}$$

where U is the urine concentration, P is the plasma concentration, V is the urine flow in ml./min., and GFR is the clearance of creatinine.

All drug doses are based on the respective salts: acetylcholine bromide and atropine sulfate. Ciba 31-531 Ba is 2-[*N*-methylpiperidyl-(4)]-3-amino-5-(4′-pyridyl)-pyrazole HCl (obtained from Ciba Pharmaceutical Co., Summit, N. J.), propranolol hydrochloride (obtained from Ayerst Lab., Inc., New York, N. Y.).

RESULTS

Direct Renal Effect of Ciba 31-531 Ba Compared to Acetylcholine—The administration of Ciba 31-531 Ba (0.1 mg./kg./min.) by infusion into the left renal artery (LRA) resulted in a diuresis and saluresis similar to acetylcholine (0.1 mcg./kg./min.) (Fig. 1). The response to direct renal infusion of 0.1 mg./kg./min. of Ciba 31-531 Ba was about 50% greater than that to the renal infusion of 0.1 mcg./kg./min. acetylcholine and was the result of an increased tubular rejection of sodium (TRF Na^+). This direct renal response to

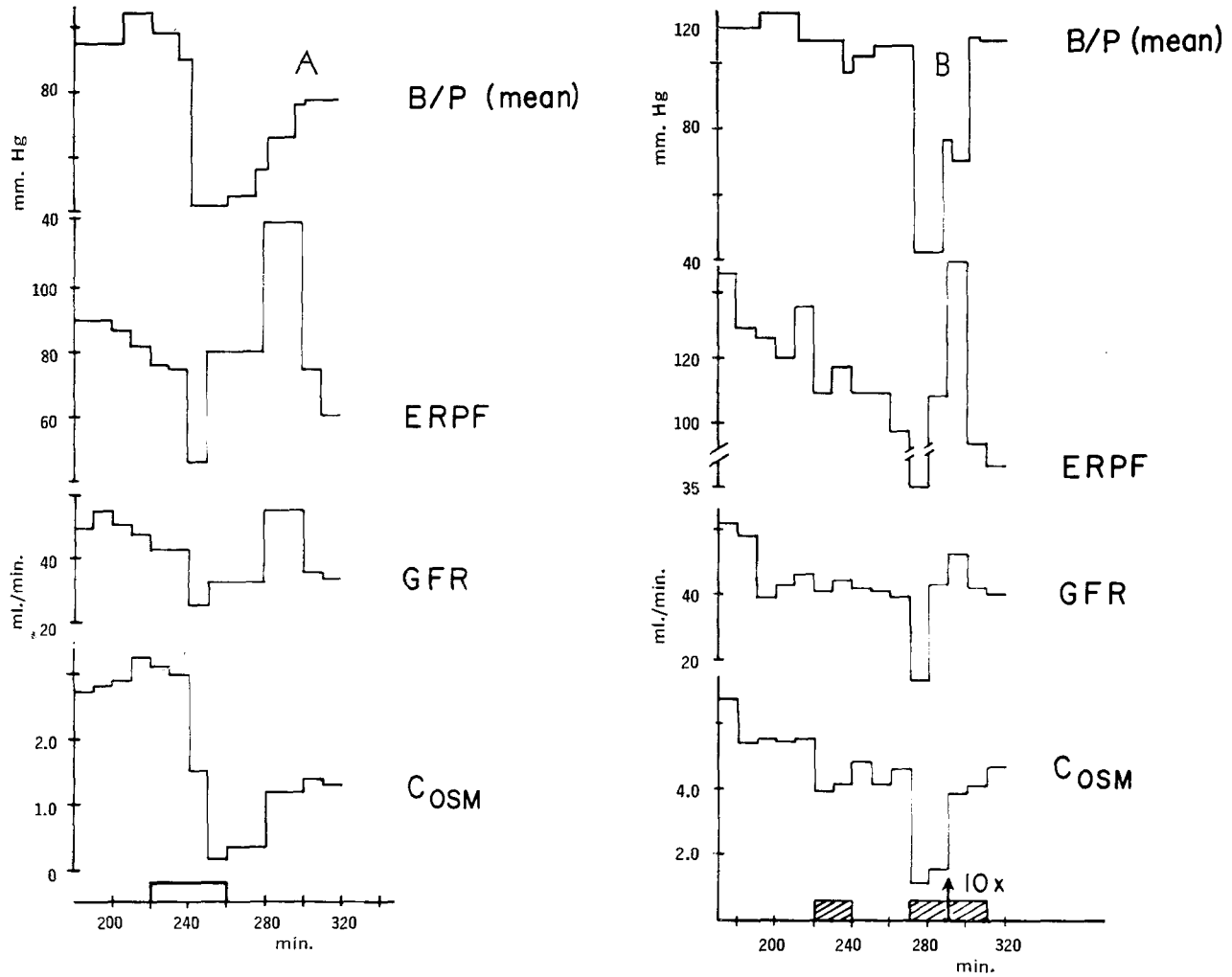


Figure 3—*Comparison of the indirect renal effects of Ciba 31-531 Ba given intravenously to a 10-kg. female dog and acetylcholine given intravenously to a 14-kg. female dog. Key:* A, □, *Ciba 31-531 Ba (20 mg./ml. i.v.);* B, ▨, *acetylcholine (1 mcg./kg./min.);* →, *atropine (1 mg./kg. i.v.).*

Ciba 31-531 Ba was not blocked by the systemic administration of atropine or propranolol (Fig. 1). The effective renal plasma flow (ERPF) was not changed during the first infusion, but it was markedly increased on the experimental side during the second 30-min. infusion. There was no change in glomerular filtration rate (GFR) during the acetylcholine infusion or during the first 10-min. infusion of Ciba 31-531 Ba. There was, however, a unilateral increase in the excretion of Na^+, K^+, Cl^-, and water. The osmolar clearance (Cosm) increased with both acetylcholine and Ciba 31-531 Ba, and the sodium contribution to the osmolality also increased with both agents.

There was no significant change in systemic blood pressure during this entire experiment.

Effect in a Dog with Marked Sodium Retention—The administration of Ciba 31-531 Ba (0.1 mg./kg./min.) LRA to a dog with plasma sodium of 168 meq./l. and oliguria resulted in a large unilateral saluresis. This animal had failed to respond with diuresis even when given 6% mannitol in a 2% NaCl solution (Fig. 2). The TRF Na^+ was 58% of filtered load. During this 10-min. infusion period the ERPF increased from a depressed 32 ml./min. to only 47 ml./min. with no change in GFR. The osmolar clearance was increased from 3.4 to 6.6 ml./min.

Indirect Renal Effects—Intravenous infusion of Ciba 31-531 Ba (20 mg./ml.) at increasing rates (total infused 400 mg.) until there was a substantial decrease in blood pressure resulted in depressed ERPF, GFR, Cosm, Na^+UV, $TRFNa^+$, and K^+UV. The infusion was discontinued and the blood pressure returned to 75% of control value with an unusual increase in ERPF and GFR during the period from 280–300 min. The osmolar clearance, however, remained depressed during the same exaggerated increase in ERPF and GFR (Fig. 3A).

The intravenous infusion of acetylcholine for two experimental periods is shown in Fig. 3B. The first period of 20 min. at 1 mcg./kg./min. i.v. did not result in a blood pressure depression. The second period of 40 min. at 10 mcg./kg./min. caused a substantial decrease in blood pressure with a decrease in GFR, ERPF, Cosm, Na^+UV, $TRFNa^+$, and K^+UV. This effect of Ach i.v. was reversed with an injection of atropine, 14 mg. i.v. (1 mg./kg.).

DISCUSSION

Evidence has accumulated that acute increases in GFR *per se* have little effect on sodium excretion (7, 8). Therefore, natriuresis requires changes in fractional tubular reabsorption. However, the performance of the renal tubules are dependent for their several clearance functions upon the blood supply to the kidney. The exact quantitative influences of renal blood flow (9), intrarenal hydrostatic pressure (10), and intrarenal distribution of blood flow (11) on sodium excretion are not understood. Some diuretics such as furosemide and ethacrynic acid, which have no marked vascular effects elsewhere other than on renal vasculature, do increase the renal blood flow in parallel with natriuresis. However, for these diuretics the increased blood flow is not a requirement for the natriuretic response (12, 13). Autonomic drugs such as acetylcholine, which have powerful vascular effects throughout the body, produce natriuresis only when they are infused into a renal artery (14). Such is the case with Ciba 31-531 Ba in these experiments. Ciba 31-531 Ba must be infused into a renal artery to obtain the saluretic

response. However, this does not mean that the saluresis produced by infusing acetylcholine, or other autonomic agents, is simply the result of changes in hemodynamics (15). McGiff *et al.* (16) have shown that the natriuresis produced by angiotensin given intravenously was independent of the magnitude of increases in blood pressure, increases of GFR, and reduction in renal blood flow. Also the degrees of renal concentration and of rates of biotransformation will determine the magnitude of systemic vascular effects. The magnitude of the systemic vascular effects will bring into play various reflex autonomic nervous activities which may enhance or antagonize direct renal effects of autonomic drugs (17). This reflex antagonism of direct renal effects is especially demonstrated by diazoxide which produces salt retention when given systemically but produces salt excretion when infused directly into the renal artery (18).

It appears from the data presented here that Ciba 31-531 Ba has both tubular and hemodynamic effects. Changes in GFR do not seem to be concerned in these phenomena. In the case of the animal with extremely high plasma sodium (Fig. 2) the GFR remained unchanged in spite of 300% increase in TRFNa^+ and 300% increase in osmolal clearance. During this 300% increase in osmolal clearance, the ERPF increased only by a factor of 47%. In this experiment the animal was retaining sodium in the face of an elevated plasma sodium. This response suggests a proximal site of action since it is improbable that a TRF Na^+ of 58% could be accounted for by a distal mechanism.

The interpretation of the renal mechanism of action remains complicated even though changes in GFR may be ignored. Saluresis was produced by increased tubular rejection of sodium accompanied by increased renal plasma flow. What the interrelationship is between these two parameters remains unknown. Neither were they always congruent (Fig. 1). Also, Harvey (19) showed that acetylcholine produced a generalized vasodilatation in the kidney along with reduced plasma extraction ratios of PAH and inulin. However, there was also an increased tubular secretion of PAH. Something more is involved than a mere shunting of the blood supply. Williams *et al.* (20) believe that acetylcholine produces its effect by an action mainly upon the renal tubules. Martino and Earley (21) postulate that the hemodynamic alterations are the chief causes for the saluresis. However, they obtained no quantitative correlation between changes in sodium excretion and changes in intrarenal venous pressure. May and Carter (15) infused arecoline into the renal portal system of hens and obtained a marked unilateral saluresis. They postulated a direct effect upon the permeability of the renal tubule since it is unlikely that the slight increases in plasma flowing through the portal system in these preparations could be the cause of the natriuresis. The authors of this article also hesitate to describe the direct renal action of Ciba 31-531 Ba as mere vasodilatation since this agent also antagonizes the effects of norepinephrine which the authors believe to cause salt retention by a tubular action (3).

Perhaps the evidence from the direct renal effects of different pharmacological agents will help in the future understanding of the relationship between tubular rejection of sodium and peritubular capillary blood flow, distribution, pressure, and permeability.

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