The Role of the Adrenergic Nervous System in the Renal Response To Acute Extracellular Fluid Volume Expansion.* (32302)

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The administration of sympatholytic agents has been previously utilized to examine the role of the adrenergic nervous system in the cardiovascular response to physiological stimuli(1). In the present investigation, the response of renal hemodynamics, urine flow, sodium excretion and osmolar clearance to acute extracellular fluid (ECF) volume expansion has been studied in dogs before and after catecholamine depletion induced by reserpine.

While studies performed after renal denervation(2) have demonstrated that the sympathetic nerves to the kidney are not essential for the natriuretic response to acute ECF volume expansion, the response after concomitant denervation of other organ systems has not been evaluated. The possible influence of the autonomic nervous system has been implicated from studies in dogs in which chronic cardiac denervation(3) resulted in a diminished diuretic and natriuretic response to intravascular volume expansion. Catecholamine depletion with reserpine afforded the opportunity to examine the effect of generalized impairment of the adrenergic nervous system on the renal response to ECF volume expansion.

Methods. Female mongrel dogs (15-25 kg) were anesthetized with intravenous sodium pentobarbital (Nembutal). The induction dose before reserpine (Serpasil phosphate) was 28 mg per kg, but after reserpine lesser amounts were required to produce comparable levels of light anesthesia as judged by preservation of corneal reflexes. Food was withdrawn 18 hours before an experiment, and the animals given 5 mg desoxycorticosterone acetate (DOCA) intramuscularly (IM). The dogs were allowed water *ad lib*. An additional 10 mg of DOCA and 2.5 units of vasopressin-tannate in oil were injected IM at least one hour prior to the experiment. At this time the inulin prime (60 mg/kg) was also administered intravenously; no p-amino-hippurate (PAH) prime was given.

Throughout each experiment a sustaining solution containing aqueous vasopressin (15-20 mμ/kg/hr), 9-alpha-fluorohydrocortisone (4 μg/ml) and sufficient PAH and inulin to maintain plasma levels of 1-3 mg and 20-30 mg%, respectively, was infused at 1.15 ml per minute. Urine collections were made by an indwelling urethral catheter. Arterial blood pressures were transmitted *via* polyethylene cannulas in the aorta, with the tip of the cannula near the origin of the renal artery, to Statham pressure transducers (Model P230b); the pressures were recorded on a multichannel Sanborn direct recorder (Model 350-1100B). Mean arterial pressures (MAP) were calculated electronically.

Rectal temperatures were recorded by means of an electronic telethermometer, and lead V4 of the electrocardiogram was continuously monitored for heart rate measurements.

Each experiment consisted of 13 periods of 15 minutes duration each. After 3 control periods, 7 infusion periods were carried out and these were followed by 3 post-infusion periods. During the infusion periods, acute ECF volume expansion was accomplished by infusing modified Ringer's lactate at 30 ml/

* This study was supported in part by John A. Hartford Grant 9893 and in part by USPHS Research Grants AM-00019-15 and AM-5100-10 from Nat. Inst. of Arthritis & Metab. Dis.

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‡ Supported in part by Research Fellowship of the Canadian Heart Foundation and Endocrine Training Grant AM-5100-10 of Arthritis and Metabolism Inst.

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min into the femoral vein. Each dog served as his own control, and identical loading studies were carried out before and after the administration of parenteral reserpine, 0.3 to 0.5 mg/kg total dose over a period of 2 to 10 days. The composition of the modified Ringer's lactate was as follows: Na 138; K 5.4; Cl 111; lactate 27.2; Ca 2.7; all mEq per liter; and 5.5 g glucose per liter. The fluid was pre-heated to body temperature and maintained at this temperature during infusion. At the end of both control and post-reserpine studies, tyramine hydrochloride was given intravenously at 100 μg/kg/min for 5 minutes and the pressure response monitored. Urine collections were terminated by washing the bladder with 50 ml of distilled water followed by 50 ml of air. Heparinized blood specimens were obtained for analyses from the femoral artery at 2.5 minutes before the midpoint of each period.

Sodium was measured by flame photometry using lithium as internal standard and osmolality was determined on a Fiske osmometer.

Inulin (C_{In}) and PAH clearances (C_{PAH}) were utilized as indices of glomerular filtration rate (GFR) and renal plasma flow (RPF). Inulin was analyzed by the resorcinol method of Schreiner(4) as adapted by Wesson(5) and PAH by an adaptation of the method of Bratton and Marshall(6).

Hematocrits were measured by the Wintrobe macrocrit method. Norepinephrine content in the kidney was analyzed by a spectro-fluorometric method(7).

The indocyanine (cardiogreen) dye dilution technique was employed to measure cardiac output(8). A high capacity Havard infusion-withdrawal pump was used for blood removal. The dye concentration was monitored with the Gilford cuvette densitometer. The integral of the dye curve was calculated by a cardiac output computer and recorded on the Sanborn multichannel recorder.

Sodium excretion per minute ($U_{Na}V$) was determined from urine sodium concentration (U_{Na}) in μEq/ml and urine flow rate (V) in ml/min. Filtered sodium (F_{Na}) was calculated as the product of plasma sodium concentration in μEq/ml (P_{Na}) $\times$ C_{In} without correction for the Donnan factor. The per cent tubular reabsorption of sodium ($\%T_{Na}$) represents $[(F_{Na} - U_{Na}V)]/F_{Na} \times 100$. Osmolar clearance (Cosm) was calculated as Uosm V/Posm where Uosm and Posm represent the urine and plasma osmolalities (milliosmols/kg).

Results. Since the pressor effect of tyramine is mediated by the mobilization of catecholamines, the degree of catecholamine depletion can be judged by the rise in blood pressure during an intravenous infusion of tyramine (9). The mean peak response to the tyramine infusion test before reserpine was 118 mm Hg and this was diminished to 10 mm Hg after reserpine. A spectrofluorometric analysis for the norepinephrine (NE) content in the kidneys of one animal also documented significant catecholamine depletion after reserpine administration in these organs. The analysis revealed 0.004 μg NE per gram of tissue and a control analysis of normal kidney contained 0.110 μg NE per gram of tissue. The control mean arterial pressure (MAP) and heart rate for all experiments were significantly lower ($P<0.05$)‖ after reserpine administration and this difference persisted during the infusion periods (Fig. 1). However, the absolute increases in MAP during acute volume expansion were similar before and after reserpine. The MAP increased 21 mm Hg during the infusion periods before reserpine and 24 mm Hg after reserpine. Twelve serial cardiac output determinations were performed in one animal during acute ECF volume expansion and the output increased from a mean control of 3.2 liters per min to a maximal value of 6.9 liters per min. In the same animal one week later after catecholamine-depletion with reserpine, the Ringer's lactate infusion produced a similar augmentation of cardiac output from 4.0 to 7.2 liters per min.

Renal clearance studies revealed comparable results before and after reserpine administrations. The GFR increased above the control values in 11 of 12 experiments during the infusion periods. The mean GFR increased from 85 ml/min to a maximal clearance of

‖ The standard "t" test for paired variates was employed to statistically compare the results during the infusion of Ringer's lactate (periods 4-10) before and after reserpine.

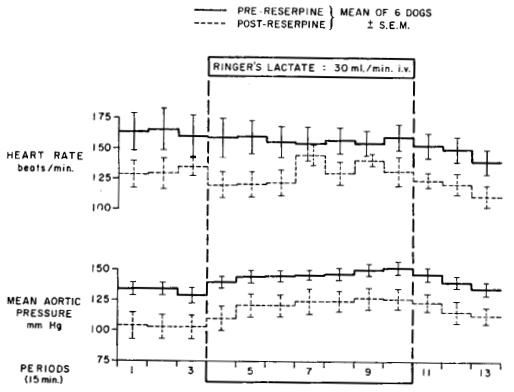
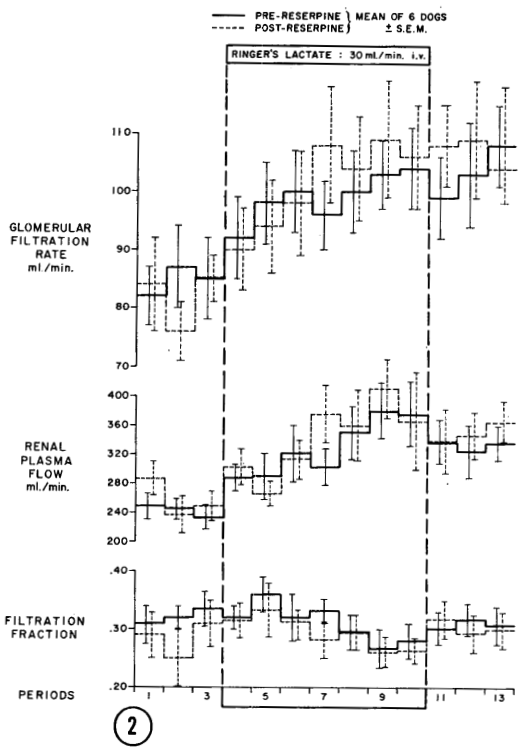


FIG. 1. Response of mean arterial pressure and heart rate to acute ECF volume expansion before and after reserpine.

104 ml/min before catecholamine depletion and from 83 to 106 ml/min after catecholamine depletion (Fig. 2). Likewise, the RPF results were not significantly different before and after reserpine (Fig. 2). In all 12 experiments the C_{PAH} increased during the infusion periods. Before catecholamine depletion the C_{PAH} increased from a mean control value of 242 ml/min to a maximal flow of 380 ml/min and after catecholamine depletion the C_{PAH} increased from 255 ml/min to 410 ml/min. With the rise in C_{PAH} the filtration fraction gradually declined during the infusion periods but was not significantly different before and after reserpine (Fig. 2).

The urine flow (V), osmolar clearance (Cosm), and sodium excretion rate ($U_{Na}V$) during the ECF volume expansion are illustrated in Fig. 3. In contrast to the GFR and C_{PAH} results, these responses of V, Cosm and $U_{Na}V$ were significantly less ($P<0.05$) after reserpine administration. Although the difference in $U_{Na}V$ could not be correlated with changes in GFR and C_{PAH}, there was a very significant correlation with MAP. In Fig. 4, the $U_{Na}V$ values for the last hours of infusion of all experiments are plotted against



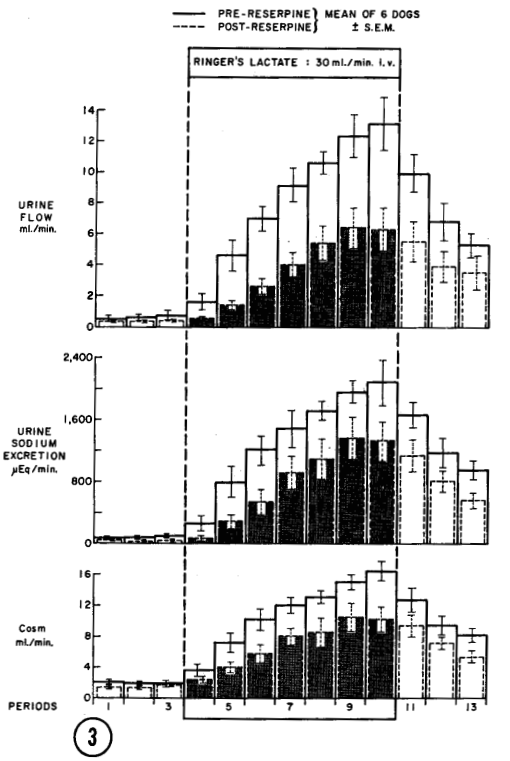
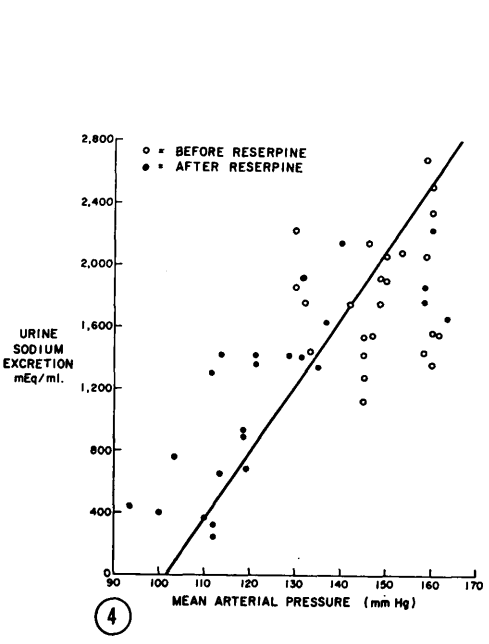


FIG. 2. Response of glomerular filtration rate, renal plasma flow, and filtration fraction to acute ECF volume expansion before and after reserpine.

FIG. 3. Response of urine and sodium excretion rates and osmolar clearance to acute ECF volume expansion before and after reserpine.



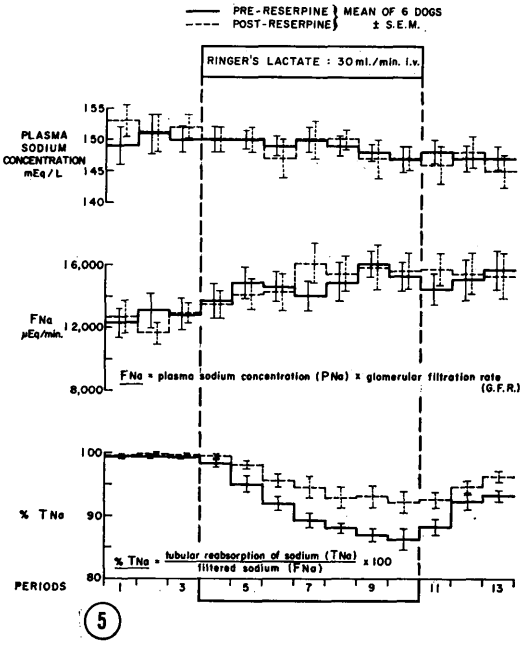


FIG. 4. Correlation between urine sodium excretion and mean arterial pressure during acute ECF volume expansion before and after reserpine. A linear regression line and correlation coefficient were calculated for the two parameters $U_{Na}V$ and MAP. The regression line was $Y = 101.894 + 0.023X$, where $X = U_{Na}V + Y = MAP$. The correlation coefficient between X and Y is 0.735. The "T" value is 7.32 with 46′ of freedom. This correlation between $U_{Na}V$ and MAP is significant at a probability of 0.001.

FIG. 5. Response of plasma sodium concentration, filtered load of sodium, and % tubular reabsorption of sodium to acute ECF volume expansion before and after reserpine.

MAP and demonstrate this correlation ($P < 0.001$).

The lack of significant difference in F_{Na} and P_{Na} during infusion periods before and after reserpine and the relatively greater fractional reabsorption of sodium ($\%T_{Na}$) ($P < 0.05$) are demonstrated in Fig. 5.

Discussion. In the control experiments before reserpine administration, acute ECF volume expansion produced marked increases in glomerular filtration rate (GFR), p-aminohippurate clearance (C_{PAH}), mean arterial pressure (MAP), urine flow (V), sodium excretion ($U_{Na}V$), and osmolar clearance (Cosm). The same dogs were studied approximately one week later after catecholamine depletion induced by reserpine but otherwise under the same experimental conditions. The depletion of the neurohumoral transmitter, norepinephrine, from the post-ganglionic efferent fibers with reserpine has been shown to impede adrenergic nerve transmission(10). The tyramine infusion test and tissue analysis for norepinephrine documented the significant degree of catecholamine depletion.

Under these conditions the role of the adrenergic nervous system in the renal response to ECF volume expansion was evaluated. The increases in GFR and C_{PAH} during the infusion were quite comparable before and after reserpine (Fig. 2), and thus the integrity of the sympathetic efferent nerves was not necessary for these renal hemodynamic responses. Since the MAP, and therefore renal perfusion pressures, were lower after reserpine, a decrease in renal arterial resistance must have accounted for the comparable GFR and C_{PAH} observed at these lower pressures.

The lower MAP after reserpine would also seem to be predominantly attributable to a decrease in total peripheral resistance. Although the MAP was lower after catecholamine depletion, the mean increase in MAP during the infusion was similar to the response before reserpine administration and the cardiac output response did not appear to be

inhibited. Moyer, Hughes, and Huggins(11) also found that the diminished arterial pressure which occurred in anesthetized dogs after the acute administration of intravenous reserpine was a result of the decrease in peripheral resistance and no consistent effect on cardiac output was demonstrated. The studies of Gilmore and Daggett(3) in chronic cardiac denervated dogs were also associated with lower arterial pressures. Since cardiac output determinations were not undertaken the relative role of diminished peripheral resistance and/or diminished cardiac output response could not be established. The authors, however, doubted a significant inhibition of cardiac function and suggested that the interference with sympathetic afferent fibers might be involved in the decreased natriuretic and diuretic responses to the intravascular volume expansion. Since there is no known effect of reserpine on sympathetic afferent nerves, the similar findings in our study of diminished arterial pressures associated with attenuated natriuretic and diuretic responses to acute ECF volume expansion imply that sympathetic efferent nerves also play an important role in these responses.

The linear correlation between $U_{Na}V$ and MAP during the last hour of the Ringer's infusion is illustrated in Fig. 4. In none of these infusion periods before reserpine was the MAP lower than 130 mm Hg and, likewise, the lowest $U_{Na}V$ was in the range of 1100 μEq/min. After reserpine there were 10 infusion periods with $U_{Na}V$ less than 1000 μEq/min and the MAP in all of these periods was lower that 120 mm Hg. Furthermore, in those experimental periods where reserpine administration did not significantly lower the MAP, the sodium excretion rates were similar to the pre-reserpine values. The comparable F_{Na} before and after reserpine (Fig. 5) would suggest that the difference in perfusion pressure affected predominantly the tubular reabsorption of sodium.

This relationship between MAP, $\%T_{Na}$ and $U_{Na}V$ in the present investigation in intact animals during acute ECF volume expansion is in agreement with previous studies in isolated perfused kidneys in dogs and rats. McDonald and DeWardener(12) found that when isolated dog kidneys were perfused at constant pressures from intact saline-loaded dogs, the greatest increases in sodium excretion occurred in those kidneys perfused at higher pressures, and these increases were unrelated to creatinine clearance, filtered sodium or renal blood flow. With stop-flow technique in the rat, Tobian, Coffee, Ferreira, and Muelin(13) have demonstrated both a primary and a preparatory influence of renal perfusion pressure on distal tubular transport of sodium. Selkurt, Womack and Daily(14) have related the natriuretic and diuretic response to elevated arterial pressures in perfused kidneys in dogs to an increased medullary blood flow and subsequent "wash out" of osmotic gradient. Recent studies by Earley, Martino and Friedler(15) have suggested, however, that the effect of perfusion pressure on tubular reabsorption of sodium may occur primarily in the proximal nephron.

In conclusion, the present study suggests that the integrity of the adrenergic nervous system plays an important role in the response of V, Cosm and $U_{Na}V$ to acute ECF volume expansion. The intact sympathetic efferent fibers maintain sufficient peripheral resistance to mediate the augmented cardiac output response of volume expansion to the kidney at a high perfusion pressure. The studies after the sympatholytic effect of reserpine resulted in diminished peripheral resistance and lower perfusion pressures in spite of similar responses in cardiac output. These lower pressures were associated with a diminished natriuretic response at comparable GFR, C_{PAH} and F_{Na} and suggested an effect on tubular sodium reabsorption. While the integrity of the adrenergic nervous system is important in the pressure-flow relationships in the kidney during volume expansion, studies during salt-loading with inflation of an aortic balloon (2) or aortic constriction(16) sufficient to lower renal pressures below control levels still demonstrated a natriuretic response and indicate that additional factors are involved. The GFR and F_{Na} were also diminished in these studies(2,16) and established that the response to acute ECF volume expansion involves predominantly a depression of tubular sodium reabsorption rather than differences

in GFR and F_{Na}. In the present study the relationship between $U_{Na}V$ and MAP during ECF volume expansion would, therefore, not unexpectedly be mediated through differences in tubular sodium reabsorption rather than differences in GFR and F_{Na}.

Summary. The role of the adrenergic nervous system in the renal response to acute extracellular fluid (ECF) volume expansion was examined in the same dogs before and after catecholamine depletion with reserpine. In the control experiments before reserpine, acute ECF volume expansion produced marked increases in glomerular filtration rate (GFR), p-amino-hippurate clearance (C_{PAH}), mean arterial pressure (MAP), urine flow (V), sodium excretion ($U_{Na}V$) and osmolar clearance (Cosm). One week later the same animals were studied under the same experimental conditions except for generalized impairment of the adrenergic nervous system secondary to catecholamine depletion with reserpine. The ECF volume expansion produced very similar increases in GFR, C_{PAH} and filtered loads of sodium (F_{Na}) before and after reserpine; however, the MAP, and thus renal perfusion pressures, were significantly lower after reserpine. Diminished urine flows (V), sodium excretion rates ($U_{Na}V$) and osmolar clearances (Cosm) occurred after reserpine and correlated closely with the level of MAP. Since the F_{Na} were comparable before and after reserpine, the effect of the lower MAP after reserpine on $U_{Na}V$ appeared to be related to a relatively greater tubular reabsorption of sodium. This study indicates, therefore, that the role of adrenergic nervous system in the renal pressure-flow relationships is an important factor in the natriuretic response to acute ECF volume expansion in dogs.

The authors wish to express their gratitude to Dr. Paul R. Draskoczy, Dept. of Pharmacology, Harvard Medical School, for norepinephrine determinations. Dr. Anthony F. Barthoomay and Miss Betsy Fuglister, Dept. of Biomathematics, Peter Bent Brigham Hospital, for statistical advice and assistance; and Mrs. Nancy Meegan and Mr. James Mowbray for technical assistance.

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Received April 19, 1967. P.S.E.B.M., 1967, v125.