The Role of the Autonomic Nervous System in Intrarenal Regulation of Renin Secretion[1] (35646)

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Since the isolation of angiotensin from dog plasma 30 years ago, investigators have devoted considerable effort to elucidating the mechanism of regulation of renin secretion. Initially, attention was directed at the role of renal arterial pressure, or pulse pressure as the regulating factor. However, more recent studies, using a refined technique for measuring renin activity, demonstrate that renin secretion can vary quite independently of arterial (1) or pulse pressure changes (2). A number of reports appear to stress the role of the autonomic nervous system (1, 3–5), body sodium or volume changes (6, 7), and intrarenal mechanisms unrelated to blood pressure changes. Because of their anatomical and functional relation to the renin secreting cells of the juxtaglomerular apparatus, the macula densa cells of the distal tubule have been considered to be involved in the intrarenal regulation of renin secretion (8–11). The sodium concentration of the tubular fluid or sodium reabsorption at this site may in some way affect renin release.

Part I of the present study was designed to examine the role of this intrarenal mechanism, namely, to see the effect of changing distal sodium reabsorption on renin secretion. It was found that the saluresis brought on by administration of chlorothiazide in the dog resulted within 15 min in an increased secretion of renin even when urinary loss of water and salt was replaced.

Part II therefore was undertaken to see whether this same effect of chlorothiazide obtained in man and whether this effect was under the influence of the autonomic nervous system.

The effect of the diuretic on peripheral renin activity was measured before and after neuronal depletion of catecholamines induced by chronic reserpine administration.

Part I. Methods. This study was carried out in 10 mongrel dogs, weighing between 10 and 15 kg. For at least 12 hr prior to the experiment, the animals received nothing by mouth except water. Under pentobarbital anesthesia (25 mg/kg) a laporotomy was performed and both renal pedicles were exposed. The right renal artery and vein were ligated. A large polyethylene catheter was advanced into the left renal vein through the stump of the right renal vein and tied into place distal to the entry of the ovarian or spermatic vein to prevent contamination of renal venous blood samples. The opposite end of the catheter was then inserted into a femoral vein. Heparin (500 U/kg) was given intravenously prior to insertion of the renal vein catheter. By means of a T-tube in the catheter and application of a clamp between the T and the femoral vein. The total renal venous effluent was periodically collected for the direct measurement of renal plasma flow (RPF) and renin activity. In the first three dogs rather than collecting the total renal venous effluent as described above, a sampling catheter was passed into the left renal vein retrograde through a femoral vein. Blood was sampled from this catheter for renin activity and PAH determinations, the latter being used in the calculation of RPF. However, in one case the catheter slipped out into the inferior vena cava and no useful data were gathered. In the other two cases shown in Table I (cases 1 and 2) it became

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TABLE I. Acute Effects of Chlorothiazide on Renal Renin Secretion in the Dog.

Case no.	GFR (ml/min)	C_{PAH} (ml/min)	ER_{PAH}	RPF C/ER_{PAH} (ml/min)	RPF Direct (ml/min)	U_{Na} (meq/liter)	$U_{Na}V$ (μeq/min)	Renin secretion (ng/min angio.)	BP (mm Hg)
1 C	17.0	41	.56	72		76	24.0	694	175/110
E	10.0	29	.36	81		127	200.0	1624	155/115
2 C	25.9	81.8	.55	148		107	30.0	709	135/100
E	9.8	38.3	.35	109		137	111.0	5885	130/90
3 C	31.0	101	.64	158	172	93	3.5	134	160/100
E	6.2	11.5	.19	61	165	114	9.5	1073	145/90
4 C	38.4	112	.63	177	129	42	11.0	1535	170/120
E	16.3	67	.26	258	125	141	9.0	3110	150/100
5 C	31.0	88	.69	128	110	17	1.4	939	130/90
E	21.0	29	.13	223	111	123	92.2	5575	135/90
6 C	22.4	67	.75	90	89	212	21.2	133	130/85
E	17.8	15	.36	42	89	150	199.5	4938	130/85
7 C	35.3	106	.62	171	160	32	5.5	1625	115/80
E	15.6	64	.32	200	172	116	72.5	3398	120/80

apparent that the administration of chlorothiazide caused a sharp drop in the extraction ratio of PAH (E_{PAH}), rendering PAH clearance and extraction calculations unreliable as a measurement of RPF. Therefore, in all other cases the direct collection method was used to measure RPF. The left ureter was cannulated with a polyethylene catheter. A femoral artery was used for blood pressure determination by means of a strain gauge transducer and Sanborn 350 recorder. A forelimb vein was used for the infusion of inulin and PAH by means of a Harvard constant infusion pump. Urine was collected to measure urine flow rate, inulin, PAH, and sodium concentrations. Before the administration of chlorothiazide, two control periods of 15 or 30 min each were obtained, depending on the rate of urine flow. During each period, blood was withdrawn from the femoral artery for measurement of inulin, PAH, renin activity, sodium, and hematocrit. Renin activity was also measured in renal venous blood. An equivalent volume of dog blood was given to replace losses and urine losses were replaced by an equal volume of normal saline. After completion of the second control period chlorothiazide (25 mg/kg) was administered into the forelimb vein. Diuresis occurred within 2 to 4 min in all dogs. Five min after injection of the chlorothiazide a 20-min urine sample was begun. At midpoint of this urine collection, samples of blood were collected as in the control state. Renin activity was measured by the method described by Boucher *et al.* (12) and expressed as (ng of angiotensin/100 ml of plasma). All determinations were performed in duplicate. Inulin concentration was measured on a Technicon AutoAnalyzer by the method of Roe *et al.* (13) and PAH was determined on the same instrument by the method of Harvey and Brothers (14). Glomerular filtration rate was determined for each period and RPF was determined from the clearance and extraction ratio of PAH or from direct measurement of RBF. Renin secretion rate was determined by the formula $\text{Renin}_{RV} - \text{Renin}_{A} \times \text{RPF}$.

Results. The data from seven dogs are shown in Table I. Three other cases are not shown. In one case, as mentioned earlier, the renal vein catheter was in the inferior vena cava and no useful data were obtainable. On two other occasions, blood pressure dropped and could not be controlled before the drug was administered. These cases were terminated at that time. In all cases, after the administration of chlorothiazide, there was a marked drop in E_{PAH}. This was felt to be the effect of competitive inhibition or poisoning of the PAH transport mechanism by chlorothiazide. Since in those cases where RPF was measured directly there was little change in RPF after drug administration, control RPF was used to calculate both control and experimental renin secretory rates in cases 1 and 2. Chlorothiazide had little effect on blood pressure. As shown in Table I, in all cases the glomerular filtration rate fell and renin secretion rose acutely after the administration of chlorothiazide. In all dogs except one, U_{Na} was increased by chlorothiazide. The control U_{Na} in that case was higher (212 mEq/liter) than any other value obtained, even after the administration of the drug (Fig. 1). In 6 of 7 dogs, sodium excretory rates ($U_{Na}V$) rose under the influence of the drug. In the case where $U_{Na}V$ did not rise, U_{Na} did rise.

Part II. The second part of this study was undertaken to examine whether autonomic nervous activity affected the intrarenal regulation of renin secretion—namely, the increase in renin release acutely in response to diuretics seen in Part I and (5, 15, 16). It was decided to use human subjects with the purpose of eventually studying this mechanism in human hypertension. It was first necessary to establish that, in man, a response to chlorothiazide could be elicited similar to that demonstrated in the dog. This response would again be examined after reducing sympathetic activity through catecholamine depletion at nerve endings by means of chronic administration of reserpine.

Methods. Six male students, between the ages of 21 and 28 years, were utilized. All were normotensive, free of renal disease by history, examination of urine, and normal blood urea and creatinine. No subjects had a family history (mother, father, siblings) of hypertension. Each subject underwent two

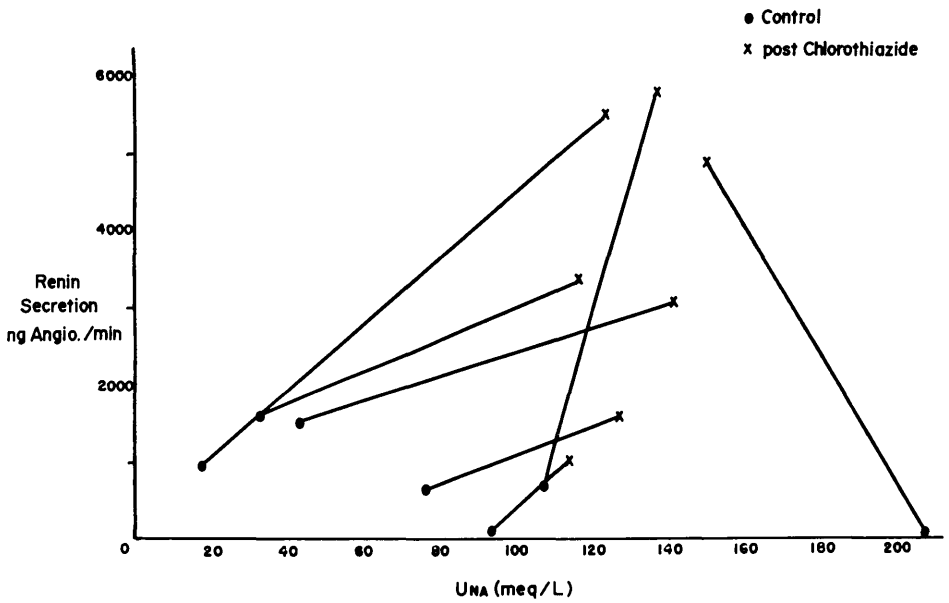

FIG. 1. The effects of chlorothiazide on sodium concentration and renin secretion.

studies, one prior to reserpine treatment and one after treatment.

For 3 days prior to the morning of the experiment each subject was placed on a 10-meq sodium diet. Meals were prepared and eaten at Georgetown University Hospital. The students maintained normal daily activity. Twenty-four hr urine collection was made from the morning of the third day to the fourth day and measured for sodium and creatinine to test the efficacy of the diet and the diligence of the subject. On the morning of the fourth day the subject was placed in the supine position for 1 hr prior to the start of the experiment. Blood pressure and pulse were then measured and a blood sample was drawn for measurement of renin, creatinine, and sodium. After urine was collected, 500 mg of chlorothiazide was administered intravenously. Blood and urine collections were repeated as in the control period at 15 and 75 min after the drug administration.

After a period of 1 to 2 weeks on a normal diet, each student was placed on 0.5 mg of reserpine, by mouth, for 8 days. All subjects tolerated this dose without significant side-effects except for occasional lassitude and stuffy feeling in the head in one case. The 10-mEq sodium diet was reinstituted on days 6, 7, and 8. On day 9, the experiment was repeated.

Creatinine was measured on the 24-hr urine to estimate glomerular filtration rate; and sodium was measured in all urines to test the effect of the diet and the diuretic. The effectiveness of reserpine in suppressing sympathetic activity was assessed by its effect on a Valsalva maneuver and on resting pulse rate.

Results. In all but two cases, the post-Valsalva maneuver bradycardia was absent. In those two cases, however, the pulse rate was significantly reduced (58 and 62 bpm) as compared to the prereserpine levels (80 and 78 bpm). As shown in Table II, reserpine had no consistent effect on glomerular filtration rate or 24-hr sodium excretion. A diuresis was seen at 15 min after chlorothiazide administration in all cases.

Table II and Fig. 2 show the effect of chlorothiazide on renin secretion and the suppressive effect of reserpine on this mechanism. In the initial study, each of the six subjects had a rise in renin levels in response to chlorothiazide at 15 min (4 cases) or 75 min (2 cases). However, after reserpine treatment, the results were quite different. In 5 cases, renin levels were unchanged or fell

TABLE II. Acute Effects of Chlorothiazide on Renin Levels in Man.[a]

No.	Peripheral plasma renin (ng of angio./100 ml) 0	15	75	GFR (ml/min)[b]	24-hr Na excretion (mEq)	Na excretion [μEq/min]; (urine flow [ml/min]) 0	15	75	BP (mm Hg)	Valsalva
1 C	290	390	160	75	10	2.1(.27)	239(26)	557(3.9)	100/70	+
R	910	870	880	83	21	15.4(.44)	530(5.0)	721(5.3)	120/70	±
2 C	45	190	380	133	26	13.5(.31)	330(2.2)	497(2.7)	130/80	+
R	570	560	590	118	35	2.4(.30)	373(2.7)	493(2.9)	110/60	±
3 C	590	1150	560	111	59	41 (.39)	607(3.3)	779(4.1)	100/70	+
R	1630	1720	870	114	83	66 (.45)	646(3.2)	912(4.0)	110/70	—
4 C	630	630	1260	115	46	8.4(.29)	477(3.1)	564(3.0)	110/60	+
R	2250	2190		102	32	6.6(.33)	319(2.2)	360(2.0)	120/70	—
5 C	910	930	1510	144	4.0	4.0(.8)	340(8.5)	789(8.3)	130/80	±
R	1130	530	560	92	21	2.5(.41)	235(2.7)	286(2.7)	140/70	—
6 C	790	1430	3290	99	29	3.9(.30)	315(2.5)	620(5.0)	120/80	+
R	560	840	1200	133	33	21 (.94)	601(7.8)	955(8.6)	100/60	—

[a] C, low sodium diet only; R, low sodium diet plus 8-days treatment with reserpine; 0, before chlorothiazide; 15 and 75 min after chlorothiazide.

[b] During 24 hr prior to the study.

after chlorothiazide; in one case, these levels rose but to a lesser degree than before reserpine (Fig. 2). These data are significant at $p<0.01$ using the sign rank test. Note in Fig. 3 that there was no difference in the prechlorothiazide levels between the control and reserpine-treated group. Using a marched-pair, sign rank test, the 75-min renin rise in the control group was significantly different at the 95% confidence level. Case number 4 was not included in the statistical analysis of the reserpine group since only two samples were obtained. However, there was no difference in the pre- and postchlorothiazide renin levels after reserpine treatment in that case.

Discussion. In 1945, Goormaghtigh (17) suggested a functional relationship between the macula densa and juxtaglomerular cells. In 1964, Thurau (18) postulated that the macula densa senses a high sodium concentration and relays this information to the juxtaglomerular cells, resulting in secretion of renin. The subsequent local formation of angiotensin II causes afferent arteriolar constriction, reduced glomerular filtration, hence conservation of sodium. The hypothesis was based on observations made during micropuncture in rat kidneys. Unfortunately, neither glomerular filtration rate, nor renin secretion could be measured in that study. Vander and Miller (11) suggested the reverse, namely that low sodium concentration at the macula densa stimulates renin secretion.

The purpose of part I of the present study was to test the hypothesis that changes in intrarenal salt handling may result in stimulation of renin release quite independent of the release brought about by salt depletion. Salt and water losses were replaced with equivalent volumes of saline.

The results of part I seem to support Thurau's hypothesis; during saluresis glomerular filtration rate fell and secretion rose consistently as would be predicted. Renal plasma flow (measured directly) was not significantly altered. The marked alterations in C_{PAH} and ER_{PAH} were most likely the result of the effects of chlorothiazide on PAH transport. Since perfusion pressure did not change during the course of the experiment, the most likely explanation for a fall in GFR and maintenance of RPF is afferent arteriolar constriction with redistribution of intrarenal blood flow.

The site of action of chlorothiazide is not

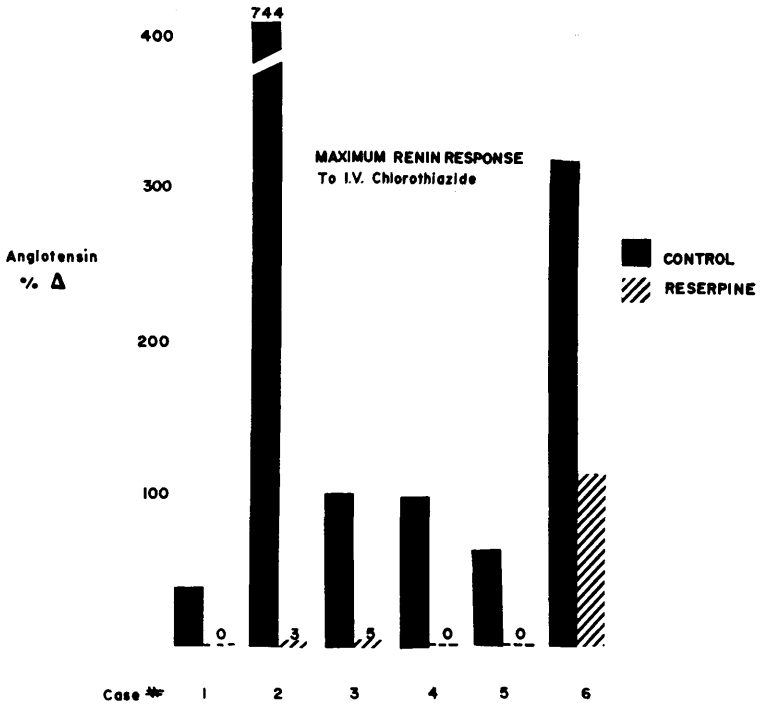

FIG. 2. The maximal acute change in peripheral plasma renin concentration following administration of intravenous chlorothiazide is shown for 6 subjects on a low sodium diet only (control); and following a low sodium diet plus 8-days pretreatment with reserpine.

completely clear. There are studies which support a site of action proximal to the macula densa (19–23) (not to the exclusion of an additional site) and others which place the site more distally (24). The most direct experiments, however, in which the sodium concentration in the early distal tubule was measured directly after administration of hydrochlorothiazide in doses equivalent to the doses of chlorothiazide used in this experiment, showed a marked rise in sodium concentration at the macula densa (20). It is likely that if sodium is the stimulus to the macula densa then, as proposed by Nash *et al.* (25), it is the rate of flux across the cell membrane which is critical. Therefore, it is possible that chlorothiazide caused a reduced flux at this site by blocking the transport mechanism. There are presently no studies which answer this question. It is clear that whatever the exact nature of the mechanism, an intrarenal regulation of renin secretion probably related to macula densa function exists. Results similar to the present study have been reported by Cooke *et al.* (16) using ethacrynic acid in dog and Meyer *et al.* (15) using furosemide in rabbit. In both studies care was taken to avoid volume or salt depletion. Although total body volume and solute were kept constant by intravenous reinfusion of urine, furosemide effected a rise in renin in 7 min. However, when animals were over expanded by saline or albumin solution prior to drug administration, no rise in renin was elicited.

The rise in renin secretion after diuretic agents has been attributed by some investigators to sodium or volume depletion, since saline replacement equivalent in volume to the urine flow reduced the elevated renin level caused by the diuretic (6, 26). In one study (6), however, the replacement was at least 75% in excess (lost 506 mEq of Na; replaced 889 mEq as saline) of urinary losses and may well have resulted in over expansion of extracellular volume. It is likely that

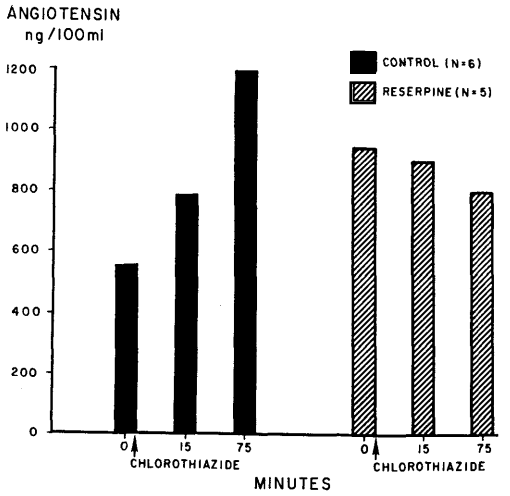

FIG. 3. Mean peripheral venous renin levels are shown for subjects during control studies and after pretreatment with reserpine. Samples were obtained prior to, and 15 and 75 min after intravenous administration of chlorothiazide.

in these studies the blocked response was due to over expansion as demonstrated by Meyer *et al.* (15).

In part II of the present study, 4 of the 6 subjects experienced a rise in renin within 15 min after the diuretic was administered. The total loss of fluid, during that time, ranged between 33 and 50 ml of urine. However, chlorothiazide was administered in 18 ml of normal saline so that it is extremely unlikely that the renin release was stimulated by volume or solute loss.

The findings in part I of this study support the theory of an intrarenal negative feedback system regulating renin release, whose stimulus is sodium flux at the macula densa. There are a number of observations to suggest that this intrarenal mechanism is modified by extrarenal factors. Salt or volume expansion seems to dampen or block intrarenal stimuli such as the response to diuretics (6, 15, 26) or to microperfusion of sodium into the macula densa area (8). A possible connecting link between extra- and intrarenal factors was considered in part II.

Although renal blood flow was not measured in these subjects, the effect of reserpine in blocking renin release after chlorothiazide administration was most likely not due to changes in intrarenal hemodynamics since there were no significant differences in the 24-hr glomerular filtration rates, salt and water excretion before and after reserpine. The dose of reserpine used in this study has been shown consistently to depress sympathetic reflexes. Employing doses of 0.5 mg/day for 5 weeks or 2.0 mg/day for 6 to 10 days, in 8 of 10 subjects the response to a Valsalva maneuver was blocked (27). In another study, all 21 subjects had significant depression of reflexes after 7 days on 0.25 mg/day (28). Reserpine inhibits neurogenically transmitted vacoconstrictor reflexes without significantly affecting adrenal release (29). Reserpine has been shown to block catecholamine storage at sympathetic nerve endings, resulting in depletion of these stores (30). Under the influence of reserpine, circulating catecholamine levels may be normal or elevated since storage is blocked and adrenal release is not markedly affected at these dose levels. Normal levels of renin were seen prior to chlorothiazide administration (Fig. 3) during reserpine administration.

Several investigators have shown that administration of catecholamine into the renal artery results in release of renin (1, 4, 31). The normal prediuretic renin levels are consistent with normal levels of circulating catecholamines. However, the nerve endings, being depleted of catecholamine, could not respond to the added stimulus of the diuretic. This implies that the acute response to diuretics operates somehow through the sympathetic nerves.

There is ample evidence that many extrarenal stimuli to renin secretion involve sympathetic stimulation. The effects of posture, reduced blood pressure, sodium depletion, and exercise on renin release can all be reduced or inhibited by cutting renal nerves, by α and β blocking agents and ganglionic blocking agents (1, 4, 5, 32–34). Keeping renal perfusion pressure constant, but stimulating the carotid baroreceptor or infusing norepinephrine into the renal artery, results in renin release (1, 4, 31). Electrical stimulation of the renal nerve results in renin release (4). In a case of autonomic dystonia, Gordon *et al.* (35) showed that both catecholamine and renin levels remain low in response to position change even though

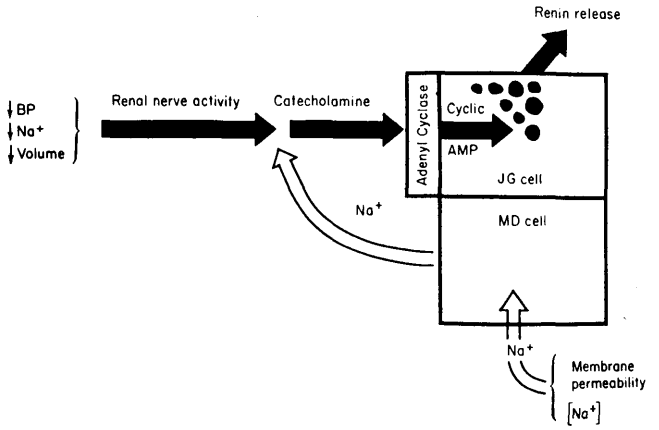

FIG. 4. Schematic representation of proposed mechanisms of renin release.

blood pressure fell markedly. After the infusion of subpressor doses of a norepinephrine:epinephrine mixture the renin levels rose in response to posture change and blood pressure was maintained.

All of the above observations support the conclusion that extrarenal stimuli to renin release involves the autonomic nervous system and operates through sympathetic renal nerves to stimulate renin release.

The present study would appear to demonstrate that the intrarenal mechanism regulating renin secretion also requires an intact sympathetic nervous system. The data would not support a simple direct axis between macula densa cell and juxtaglomerular cell. They require the involvement of renal nerves being interposed (at least functionally) between the two cells, perhaps playing a permissive role.

In another series of experiments done in this laboratory, it was found that catecholamine turnover rate from nerve endings in the rat heart was markedly influenced by the sodium concentration of the perfusing medium (36). Reducing sodium content (by replacement with lithium) in the medium perfusing the coronary circulation resulted in a more rapid release of catecholamine from the heart. The ratio of norepinephrine to intraneuronal and extraneuronal metabolite, however, did not change, indicating that sodium may affect the ability of the neurone to store norepinephrine in the granules. Such an effect of sodium on catecholamines release at nerve endings could have direct application to the mechanism of renin release in the kidney. Changes in sodium flux within the macula densa might alter the rate of catecholamine release from the nerve endings in the juxtaglomerular cells. This change in catecholamine release would then effect a change in renin release.

There are at least two observations which support the concept that the link between catecholamine release and renin release is cyclic AMP. In one study (5), although α and β blocking agents reduced renin release, these agents could not block the effect of theophylline, which increased renin release. Theophylline is known to block the breakdown of cyclic AMP by inhibiting phosphodiesterase activity. The other report demonstrated that incubating kidney tissue slices with cyclic AMP resulted in renin release into the medium (37). Figure 4 is a scheme of a possible explanation of the interrelationship of intra- and extrarenal factors regulating renin release. Extrarenal factors known to stimulate renin release such as reduction in blood pressure, hypovolemia or sodium depletion, exercise, posture change, etc., operate by stimulating the renal nerves (effects can be blocked by cutting the renal nerve) which release catecholamine. Catecholamine, in turn, acts on some receptor (which may be adenyl cyclase generating cyclic AMP) which stimulates renin release. The intrarenal mechanism is initiated by changes in ionic flux at the macula densa site. This change in flux affects

the ionic composition around the sympathetic nerve ending or receptor site in the juxtaglomerular cell resulting in a change in catecholamine release and, finally, renin secretion.

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