ABSTRACT

Experiments were undertaken to determine effects of d-propranolol and renal denervation on furosemide induced renin secretion in the anesthetized dog. D-propranolol possesses only membrane stabilizing properties whereas the l-isomer produces beta adrenergic blockade. To effectively separate the vascular and macula densa mechanisms of the juxtaglomerular apparatus, non-filtering kidneys were produced by combining 2.5 hours of renal ischemia with ureteral ligation. In some animals, renal denervation was accomplished by relocating the non-filtering kidney into the neck during the 2.5 hour ischemic interval. Administration of d-propranolol in a priming dose of 1 mg/kg i.v. followed by an intravenous infusion of 1 mg/kg/hr decreased renin secretion in both the filtering and non-filtering kidney. Subsequent furosemide injection (5 mg/kg i.v.) failed to increase renin secretion in the non-filtering kidney while renin secretion was significantly increased in the filtering kidney. Similarly, following the infusion of lidocaine into the renal artery of the non-filtering kidney (1 mg/kg/hr), furosemide did not alter renin secretion. In the denervated non-filtering kidney, furosemide, in a dose of 5 mg/kg i.v. increased renin secretion and decreased renal resistance. Treatment with d or d,l-propranolol decreased renin secretion in 5 out of 6 denervated, non-filtering kidneys. Following propranolol treatment, furosemide failed to increase renin secretion. These results indicate that furosemide stimulates renin secretion at both

the vascular and macula densa sites. The ability of these mechanisms to alter renin secretion are independent of each other. Also, the ability of d,l-propranolol to decrease renin secretion may be partially due to the membrane stabilizing activity of the d-isomer. The data suggest that the sympathetic nervous system acts independently of the vascular receptor and the macula densa receptor.

CONTROL OF RENIN SECRETION: EFFECTS OF D-PROPRANOLOL AND RENAL DENERVATION ON FUROSEMIDE INDUCED RENIN SECRETION IN THE DOG.

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INTRODUCTION

Three primary mechanisms have been described in the control of renin release from the kidney: the macula densa mechanism located in the distal tubule, the baroreceptor mechanism in the afferent glomerular arteriole and the sympathetic innervation of the juxtaglomerular cells. Recently, the stimulation of renin secretion by the diuretic, furosemide, has been shown to occur through both the macula densa and baroreceptor mechanisms (37). The macula densa receptor has been shown to be responsive to alterations in sodium or possibly chloride transport (9, 93, 145). Furosemide, which blocks the transport of sodium (chloride) in the ascending limb, may alter renin secretion at the macula densa receptor by this mechanism.

The vascular receptor may be stimulated by changes in afferent arteriolar wall tension or changes in the vascular transmural pressure gradient (128). Furosemide has been shown to stimulate renin secretion on the vascular side of the juxtaglomerular apparatus by vasodilatation of the baroreceptor (37).

The role of the sympathetic nervous system in the control of renin secretion is unclear. There are two mechanisms by which the sympathetic nerves may regulate renin secretion. Since direct innervation of the juxtaglomerular apparatus has been demonstrated (11), changes in sympathetic tone may modify renin release. However, renin secretion may also be altered through modulation of the vascular receptor by the sympathetic nervous system. To further define the role

of the vascular receptor and renal innervation in the control of renin secretion, experiments were conducted utilizing the non-filtering kidney model developed by Blaine et al. (19). The results of this study demonstrate that the sympathetic nervous system may act independent of the renal vascular baroreceptor and the macula densa receptor in the control of renin secretion.

HISTORICAL REVIEW

The first evidence that hypertension may be caused by the kidney was reported by Richard Bright in 1828. The hypertensive substance was demonstrated by Tigerstedt and Bergman in 1898 when they found that crude extracts of rabbit kidney injected into anesthetized rabbits produced a pressor response (135). They named this pressor substance renin. This evidence was disputed for some time until Goldblatt demonstrated that renal artery constriction produced a reproducible form of diastolic hypertension (46). The renal juxtaglomerular cells were described by Goormaghtigh (49) and since the Goldblatt type of hypertension produced an increase in the granularity of these cells, he suggested that these cells were secreting renin. In 1939, Page (108) and Braun-Menendez (21) reported that renin was not the pressor substance but acted as an enzyme, which released the pressor substance, angiotensin, from a circulating plasma globulin.

Possible renin containing cells were first described by Ruyter (118) as granulations of the afferent arteriole approaching the glomerulus. These cells of granulation were later expressed by Goormaghtigh (49) as the juxtaglomerular (JG) cells. Recently, the juxtaglomerular apparatus (JGA) has been expanded to include the macula densa and polkissen cells.

The JG cells have been reported by electron microscopy to contain an abundance of mitochondria, endoplasmic reticulum with ribosomes, golgi elements and osmophilic granules bounded by

distinct membranes (11). Since myofibrillar elements are frequently observed within these cells, it has been suggested that they are modified smooth muscle cells. Goormaghtigh proposed that these JG cells were the source of renin since he had observed hyperplasia in this granulated area in the hypertensive rabbit and dog (48, 50). Subsequent studies demonstrated that hypergranulation of the JG cells occurred with prolonged hypoxia (105), sodium deficiency (58), hyponatremia (114) and adrenal insufficiency (57).

There is little doubt that renin is concentrated in the JG apparatus. However, the primary site of the synthesis of renin has been disputed between the macula densa and the JG cells. McManus (91) has pointed out that the golgi apparatus of the macula densa actually faces the afferent arteriole of the glomerulus. Thus, he postulated that renin may be formed in the macula densa and transported to the JG cells for storage and release. However, the micropuncture studies of Cook have shown that the JG granules actually contain renin (34). Similarly, Robertson et al. demonstrated that cultures of JG cells may produce renin (117). This does not show that renin cannot be synthesized by the macula densa, but presently, the macula densa is not thought to be the primary source of renin production.

Two specific extrarenal sources of renin have been shown in the submaxillary gland of the mouse (157) and the uterus of the rabbit (42, 53). However, these extrarenal sources do not appear to contribute significantly to the total plasma renin concentration of these species. Skeggs et al. have demonstrated a pseudorenin that can generate angiotensin I from the

synthetic tetradecapeptide renin substrate or from purified hog renin substrate (125). This enzyme is distinguished from renin normally detected in the plasma because its maximal activity is demonstrated at a very low pH. Low pH renin activity has been found in several different tissues and in the plasma. Pseudorenin is especially concentrated in human serum.

The purification of renin was first conducted by Haas, Landstrom, and Goldblatt in the hog kidney (54). The half-life of the enzyme in the circulation of the rat is about 15-20 minutes (81, 165). Renin has been shown to be excreted in the urine of humans (23) and thus, may be partially filtered by the glomeruli. However, urinary renin is significantly enhanced following tubular damage and in patients with tubular disease, suggesting that considerable tubular reabsorption of renin exists (116, 111). Recently, the liver has been recognized as the major site of renin inactivation (59, 119).

There is no known single physiological action of renin. Plentyl et al. (115), demonstrated that the cofactor necessary to elicite the pressor response of renin was a protein characterized primarily in the α-2 globulin fraction of the plasma. A tetradecapeptide was then purified by Skeggs et al. (124) and this polypeptide was shown to yield angiotensin upon incubation with renin. Thus, the effects of the enzyme may only be expressed in terms of its ability to generate angiotensin. In this same study, the action of renin on its substrate, angiotensinogen, was determined. Renin was shown to act on the tetradecapeptide by splitting the dileucyl bond from the carboxyterminal end, leaving the angiotensin I decapeptide

as the product from the N-terminal end. In most naturally occurring situations, renin substrate concentrations are low such that first order reaction kinetics prevail (102). Thus, changes in substrate concentration may alter the rate of angiotensin production. However, the Michaelis-Menten constant (Km) may vary considerably in different species.

In 1954, Skeggs et al. (126) separated two forms of angiotensin by countercurrent distribution. One product was shown to be the decapeptide angiotensin I and the second, an octapeptide, angiotensin II. Angiotensin II is formed by the removal of the terminal histidylleucine from angiotensin I. It was also shown that angiotensin I alone exhibited no significant vaso-constrictor properties in the rabbit aortic strip or the rat kidney. The angiotensin II however, was highly active in its pressor response. This evidence suggested that an enzyme was present in the plasma which was responsible for the conversion of angiotensin I to angiotensin II.

The plasma converting enzyme was first isolated from horse plasma (83). Similarly, Lentz et al. identified the presence of the dipeptide histidylleucine in the reaction between angiotensin and plasma converting enzyme. Later, Ng and Vane (103) and Biron and Huggins (15) showed that angiotensin I was converted to angiotensin II in a single passage through the lungs. This rapid conversion explained the immediate rise in blood pressure seen following the intravenous injection of angiotensin I. Converting enzyme has also been identified in the heart, aorta, ileum and liver. A particularly

significant amount of converting enzyme activity has been demonstrated in renal tissue (10, 30, 107).

Angiotensin II is both a potent vasoconstrictor and a hormone which stimulates aldosterone secretion from the adrenal cortex. The pressor action has been attributed to arteriolar or precapillary vasoconstriction by direct observation of arteriolar contraction upon angiotensin infusion. Venules and capillaries are unaffected. The ability of the hormone to constrict the arteriole is due to a direct action upon the smooth muscle cells. This has been shown in vitro, where autonomic influences are eliminated. It has been suggested that angiotensin elicits the contraction response in smooth muscle cells by a direct effect upon the cell membrane (5). Turker et al. substantiated this work by blocking this angiotensin response with ouabain (139). Several studies have suggested that angiotensin may facilitate the vasoconstrictor action of the sympathetic nervous system (40, 90, 110, 166, 121). However, following bilateral nephrectomy blood pressure homeostasis is unaltered. Angiotensin has been demonstrated as a potent ganglionic stimulant (84) and this stimulation is blocked by both atropine and hexamethonium (73).

The vasoconstrictor action of angiotensin II also has a significant effect upon the kidney. Angiotensin II administration produces an immediate decrease in renal blood flow accompanied by a proportionate decrease in glomerular filtration rate (GFR) (20, 80). Upon continued infusion of moderate doses, the GFR returns toward the normal suggesting that a tachyphylaxis occurs. A similar tachyphylaxis has been

reported on the systemic pressor response of angiotensin II (25, 87, 106). It has been demonstrated by McGiff that the depression of GFR may be related to catecholamine release since this decrease was abolished by pretreatment of the kidney with reserpine or guanethidine (89). The renal vasoconstrictor effect does not appear to be catecholamine related since renal denervation did not alter the pressor action of angiotensin II on the kidney. Thurau et al. have postulated that intrarenal release of angiotensin II may decrease renal blood flow, thus altering GFR (133). This theory has been supported by Bailie et al. (9, 10) by the identification of renin in the renal lymph of the dog.

The intrarenal release of AII may also be involved in the distribution of blood flow in the kidney. Locally generated AII has been shown to decrease medullary and inner cortical blood flow while exogenously administered AII does not (64, 65). The micropuncture studies of Thurau et al. (133) demonstrated that changes in the load of sodium and chloride at the macula densa produce changes in renin concentration at the JG apparatus. Recently, it has been reported by McGiff et al. (64, 65) that the vasculature of the inner cortex may be sensitive to both prostaglandin E-2 and the kinins. Administration of the converting enzyme inhibitor, SQ 20,881, produced a shift in blood flow to the inner cortex of the kidney (8). Inhibition of converting enzyme would further inhibit AII production as well as the degradation of bradykinin. This shift in blood flow may be due to the activity of these vasoactive substances.

A dose related effect of angiotensin II has been shown on sodium and water excretion. An antinatriuretic and antidiuretic effect has been shown by the infusion of AII in a dose of 0.003 - 0.1 mg/kg/min . This sodium and water retention has been related to the renal vasoconstriction and decrease in GFR which occurs at these doses (129). However, higher doses of 0.06 - 0.13 mg/kg/min of AII produced a diuresis and an increase in sodium excretion. This effect at the higher dose has been explained by two possible mechanisms: alterations in post-glomerular circulation may produce natriuresis or direct inhibition of tubule sodium transport. Vander has shown by stop-flow technique that inhibition of sodium reabsorption occurs at the distal tubule (144). Studies on the infusion of AII into the renal portal system of the chicken indicate that an ipsilateral natriuretic and diuretic effect is observed (79). It has been demonstrated that AII inhibits the uptake of sodium in renal tissue slices (85) however, no effect of AII was seen on the sodium transport system of the toad skin or bladder (12, 38).

Angiotensin II is rapidly metabolized in the plasma and has a biological half-life of about 1-3 minutes. It has been demonstrated that 70% of the hormone is destroyed in one pass through the liver (4, 16, 66, 92) or renal (16, 66) vascular beds. However, the pulmonary circulation has no effect on the metabolism of angiotensin II (15, 103).

At least two different angiotensinases in the plasma have been characterized as specific proteolytic enzymes of AII.

Angiotensinase A has been described (74) and an endopeptidase similar in activity to chymotrypsin has been reported by Pickens et al. (113). In addition to these specific angiotensin II metabolizing agents, angiotensin II may be degraded by any one of several proteolytic enzymes including amino and carboxy-peptidases, chymotrypsin, trypsin, pepsin and papain (22).

From this discussion, the renin-angiotensin system may be summarized as the following:

Renin Substrate - alpha 2 globulin $\xrightarrow{\text{Renin}}$ Angiotensin I

Angiotensin I $\xrightarrow{\text{Converting Enzyme}}$ Angiotensin II

Angiotensin II $\xrightarrow{\text{Angiotensinase}}$ Angiotensin II Metabolites

Thus, by obtaining a more thourough understanding of the control of renin secretion, a more efficient method of controlling the renin-angiotensin system may be developed.

Three mechanisms have been recognized in the control of renin release: a vascular mechanism which may be located in the afferent glomerular arteriole, a tubular mechanism in the macula densa region of the distal nephron and a sympathetic mechanism which includes an adrenergic receptor possibly located in the afferent arteriole.

The early experiments of Goldblatt et al. (46) produced the hypothesis that renal ischemia was the primary mechanism for the stimulation of renin release. The possibility that hypoxemia due to decreased renal blood flow was actually stimulating renin release was then studied by several investigators. Huidobro and Braun-Menendez demonstrated that the

intake of 7% - 8% oxygen and 5% carbon dioxide produced no change in renin release (63). Reduction of the arterial oxygen saturation from 96% to 56% and the infusion of venous blood into the renal artery indicated no change in renin release (26, 127, 128). In these experiments, renal blood flow was unchanged. This evidence confirmed that renal hypoxia was not a stimulus associated with renin release and the work of Skinner, McCubbin, Page and Kolsteadt (127, 77) demonstrated that a vascular mechanism existed in the control of renin secretion.

The baroreceptor hypothesis was first proposed by Tobian et al. in 1959 (137). By altering the perfusion pressure of the rat kidney, Tobian recognized changes in JG granulation. Since the JG cells are located in the wall of the afferent arteriole, it was suggested that these cells were responding to changes in the degree of stretch of the arteriolar wall. However, changes in renal perfusion pressure, glomerular filtration rate or renal blood flow may also have altered the JG granulation in these experiments. Skinner, McCubbin and Page reported that suprarenal aortic constriction increased renin release without decreasing renal blood flow (127). These same authors then increased the renal interstitial pressure by compression of the kidney with an oncometer and observed an increase in renin release (127). Both suprarenal aortic constriction and compression of the kidney decrease the renal perfusion pressure. These experiments led to the hypothesis that changes in renal perfusion pressure may also alter the secretion of renin.

The isolation of the vascular receptor was provided by Blaine et al. (17, 18, 19). This vascular receptor was isolated by the production of a denervated non-filtering kidney in an adrenalectomized animal. The non-filtering kidney eliminates any alterations in sodium load to the macula densa. Subsequently, renal denervation and removal of the adrenal glands eliminates the affects that the sympathetic nervous system or circulating catecholamines might have on renin secretion. In this model, the vascular receptor is isolated. In the denervated, non-filtering kidney, Blaine et al. (19) demonstrated that both hemorrhage and suprarenal aortic constriction produced an increase in renin secretion. These experiments indicate the presence of a functional vascular receptor within the kidney. Recently, it was shown that increased ureteral pressure and decreased renal arterial pressure, increase renin release. Conversely, increased renal arterial pressure in the normal animal as well as superimposed upon ureteral occlusion will decrease renin release (32, 61, 72). This evidence substantiates that changes in renal perfusion pressure may stimulate the vascular receptor to alter renin release. The location of this vascular receptor was examined by the experiments of Witty et al. (161). The smooth muscle relaxant papaverine decreased the renin releasing response of the vascular receptor to hemorrhage, chronic sodium depletion and thoracic caval constriction (19, 52, 162). This evidence supports the existance of a vascular receptor and since papaverine acts on the vascular smooth muscle, the evidence suggests that the receptor is located within the

afferent arteriole.

These experimental maneuvers (thoracic caval constriction, chronic salt depletion and hemorrhage) which have been shown to increase renin secretion may all be associated with increased renal vascular resistance. The constriction of the afferent arteriole may occur prior to the JG cells or at the site of the JG cells. It was first reported by Skinner, McCubbin and Page (127, 128) that renin secretion may also be stimulated by a decrease in renal vascular resistance. In this study, suprarenal aortic constriction increased renin release without a further decrease in renal blood flow. Renal resistance decreased in this experiment due to renal autoregulation. Since renal blood flow was unaltered, changes in sodium load at the macula densa were minimal.

Regional changes of blood flow within the kidney have also been shown to alter renin release presumably at the vascular receptor. Abe et al. (3) reported that only when renal perfusion pressure was reduced below the range of autoregulation did renal blood flow shift from the outer to the inner cortex and renin secretion increase. Elevation of the venous pressure also produced increased inner cortical blood flow associated with decreased outer cortical blood flow and increased renin release (75, 76). The data suggests that below the range of autoregulation, blood flow shifts to the inner cortex and renin secretion increases.

The stimuli to which the vascular receptor may be sensitive has been suggested by Vander (141) as one or a combination of

three mechanisms: 1) a decrease in the transmural pressure gradient 2) a decrease in the intraluminal pressure of the arteriole at the level of the JG cells or 3) a decrease in the tension within the arteriolar wall of the JG cell region. According to Laplace's Law the tension is equal to the product of the transmural pressure gradient and the radius of the arteriole. Thus, these factors may be acting alone or in combination with each other to alter renin release.

Several different inputs may be responsible for affecting one of these mechanisms. Davis and Freeman (39) have suggested that these inputs may include changes in the afferent arteriolar diameter, changes in the transmural pressure gradient, changes in renal arteriolar tone by the sympathetic nervous system, intrinsic myogenic factors involved in renal autoregulation or alterations in the elastic components of the vessel wall.

The macula densa, was first associated with the JG cells by Goormaghtigh (47). In 1964, Vander and Miller (147) provided experimental evidence that the macula densa may alter renin release. It was demonstrated that the increase in renin release produced by aortic constriction could be prevented by the administration of diuretics. The hypothesis was then proposed that a decrease in sodium load to the macula densa produces an increase in renin secretion. This result has been substantiated by the fact that decreased sodium intake, volume depletion, hyponatremia and decreased GFR all act as a stimulus to increase renin release (44, 146, 147). Similarly, extracellular fluid volume expansion and sodium

loading produces a decrease in renin secretion (88, 138). The decrease in renin secretion is associated with an increase in filtered sodium load.

This view has been opposed by the micropuncture studies of Thurau and associates (132, 134). In these experiments, retrograde perfusion of the macula densa with hypertonic sodium chloride produced a collapse of the proximal tubule and an increase in renin release from the JG cells. Recently, Thurau has shown that this effect is specific to the sodium ion. It was suggested that the increased sodium concentration produced an increase in renin release from the JG cells. Local formation of angiotensin II was theorized as the humoral agent responsible for afferent arteriolr constriction and a subsequent decrease in GFR resulting in the collapse of the proximal tubule. Others have also supported the hypothesis that increased sodium load produces increases in renin release. Perfusion of the loop of Henle by micropuncture with isotonic sodium chloride decreased single nephron glomerular filtration rate (SNGFR) (120). Plasma renin activity increased in rabbits treated with furosemide even though sodium and volume depletion were prevented (93). It was suggested that furosemide increased the concentration of sodium at the macula densa by decreasing sodium reabsorption in the ascending limb, thus, increasing renin secretion. Chlorothiazide, which acts on the distal nephron failed to alter renin release (35). The hypothesis proposed by Thurau however, is questioned since Vane was able to show only a small amount of conversion of

AI to AII within the kidney (150). Following ethacrynic acid administration, Freeman et al. (45) observed a decrease in renin secretion associated with an increase in sodium concentration at the macula densa (45). Recently, the infusion of ouabain has been shown to blunt the renin response to ureteral occlusion or aortic clamping (33). It was suggested that this blunting was due to an increase in distal tubular sodium concentration.

Infusion of sodium chloride into the renal artery was reported by Nash et al. (100) to decrease renin release induced by ureteral occlusion. It was suggested that the macula densa cells were responding to decreased sodium transport to produce an increase in renin secretion. Vander and Carlson have supported this proposal as an explanation of the increase in renin release following furosemide administration (145). Furosemide may have a direct action on the macula densa to decrease sodium transport.

Tubular calcium may also be involved in the regulation of renin secretion at this site. The presence of calcium in the perfusion fluid directed into the loop of Henle produced decreases in SNGFR (66, 29). It was suggested that distal calcium delivery may function on a macula densa - glomerular feedback mechanism in controlling GFR.

Plasma potassium loading was shown to decrease plasma renin activity in the dog while potassium depletion increased the PRA (1, 2). Similar results have also been shown in normal subjects and hypertensive patients (151). The mechanism

of action by which potassium produces this effect has been suggested by Vander (142) as tubular in origin. Similarly, experiments by Shade et al. (122) in the non-filtering kidney have demonstrated that the vascular receptor is not affected by potassium.

Evidence for a chloride pump in the ascending loop of Henle has also been presented (28, 78). Wright and Persson (163) have demonstrated that electrical potential differences in the distal tubule favor the movement of a negatively charged ion such as chloride. Therefore, the possibility that the macula densa cells may be sensitive to changes in chloride load or transport exists.

Thus, the evidence established, indicates that a distal tubule site may be sensitive to renal tubular sodium and this site appears to be located in the macula densa region. However, it is not entirely clear whether renin secretion is stimulated at this site by increases in sodium load or decreases in sodium transport. Recent evidence also suggests that the macula densa may monitor calcium load or chloride transport as well.

The innervation of the kidney consists primarily of sympathetic fibers. In 1964, the electron microscopic studies of Barajas (11) demonstrated that nonmyelinated nerve fibers were positioned in the region of the afferent and efferent arterioles. A histochemical fluorescence technique for biogenic amines was utilized by Wagermark et al. (152) to show that these sympathetic nerve terminals contained granules. The macula densa region was also reported to contain some

sympathetic innervation (56), although granules were not located in this region (152). The proximal and distal tubules have also been described as having both adrenergic and cholinergic fibers in close contact with their basement membranes (98).

The renal innervation is thought to originate primarily from the sympathetic nervous system. Studies by Silverman and Barajas (123) demonstrated that treatment with reserpine produced a depletion of granulation within the nerve terminals. Subsequent assay by the monoamine-specific fluorescence technique confirmed that depletion of renal catecholamine levels had occurred. Thus, direct sympathetic innervation of the JGA is verified.

Direct stimulation of the renal nerves has been shown in several studies to produce an increase in plasma renin activity (36, 70, 86, 143). The removal of the sympathetic nerve response by various methods has also been shown to have an affect upon renin release. Ganglionic blockade and local anesthesia of the nerves blocked the renin response to mild hemorrhage where perfusion pressure and renal blood flow were unaltered (27). An increase in sympathetic nerve activity has been implicated by Weber et al. as a mechanism for renin release in nonhypotensive hemorrhage (156). Similarly, renal denervation produced a blunting of the renin response to acute suprarenal stenosis (164). In acute salt depletion however, Vander and Luciano (146) have shown that the autonomic nervous system is not necessary for the stimulation of renin release. This evidence suggests that the renal nerves are mediating the response to acute salt depletion and hemorrhage.

The sympathetic nervous system may also affect renin secretion by altering the vascular tone. This modulation of the baroreceptor sensitivity may occur directly at the JG cells (137). The possibility also exists that the modulation is catecholamine mediated through a specific adrenergic mechanism.

Several experiments have shown that chronic renal denervation produces a decrease in plasma renin activity (131, 136, 14). However, it has been demonstrated by Mogil et al. that renal denervation had no effect on renin activity in salt depleted animals (95). It was reported by Gotshall et al. (52) that plasma renin activity increased in a similar manner in sodium depleted dogs both before and after renal denervation. These experiments demonstrate that the renal nerves are involved in the renin response produced by various stimuli. However, other renal mechanisms also will stimulate renin release in the absence of the sympathetic nervous system.

Recently, evidence has accumulated implicating the central nervous system in the control of renin release. Stimulation of the central gray stratum of the midbrain (140) and the medulla oblongata (109) produced an increase in renin activity. Renal hemodynamic changes were associated with this increase however, renal denervation either blunted or abolished the response. Muow et al. (96, 97) has suggested that the brain ventricular areas may be sensitive to changes in the sodium concentration of the cerebrospinal fluid (CSF). Perfusion of the CSF with hypotonic saline solutions produced

an increase in PRA and a decrease in sodium excretion.

The accumulation of evidence demonstrating that renal nerve stimulation increases renin release has led to studies concerning the effects of catecholamines on renin secretion. The infusion of epinephrine and norepinephrine in dogs was shown to increase the PRA (143, 155). Norepinephrine produced an increase in renin release in the rat and this increase was not blocked by phenoxybenzamine (148, 149). Phenoxybenzamine, however, prevented any further changes in renal hemodynamics. Experiments by Johnson et al. (70) in the non-filtering kidney, have demonstrated a direct effect of norepinephrine on the JGA. The increase in renin secretion produced by epinephrine was blocked by vasodilatation with papaverine in the non-filtering kidney, however, the renin response to norepinephrine was unaltered. The data provide further evidence for a direct action of the sympathetic nervous system on the juxtaglomerular cells.

It was reported by Winer et al. (159) that increases in PRA due to diazoxide, theophylline and ethacrynic acid were inhibited by both alpha adrenergic blockade and beta adrenergic blockade. This experiment, coupled with the effect of catecholamines on renin secretion, suggested that an adrenergic mechanism was involved in the sympathetic control of renin release. Several investigators then demonstrated that the infusion of isoproterenol increased renin secretion (7, 71, 82, 149) and this increase was blocked by d,l-propranolol (7). In the experiments of Vandogen et al. (149), isoproterenol induced renin secretion was unaffected by phenoxybenzamine.

The alpha adrenergic agonist methoxamine did not alter renin secretion. This evidence indicates that an intrarenal beta adrenergic receptor is involved in the control of renin secretion. Recently, Johnson et al. (69) have shown that intrarenal administration of low doses of d,l-propranolol in sodium depleted dogs with a denervated, non-filtering kidney decreased renin secretion without any effect upon renal or systemic hemodynamics. This low dose of d,l-propranolol was ineffective when administered systemically. The data were interpreted to suggest that an intrarenal beta adrenergic receptor exists and is located within the membrane of the JG cells. Studies by Winer et al. (158, 160) have shown that cyclic AMP will stimulate renin release and this stimulation is blocked by both propranolol and phenoxybenzamine. These authors have suggested that cyclic AMP may be an intracellular mediator of renin release that is activated following adrenergic stimulation within the cell membrane.

RATIONALE

This study was undertaken to further define the role of the vascular receptor and the sympathetic nervous system in the control of renin secretion. The d-isomer of the beta adrenergic blocking drug, d,l-propranolol, contains only membrane stabalizing properties similar to local anesthetics. D-propranolol was used to inhibit the activity of the vascular receptor in the afferent arteriole. The diuretic furosemide, which decreases renal resistance, was used to stimulate the vascular receptor to release renin. The vascular receptor was effectively isolated by removal of the sympathetic nervous system and macula densa mechanism in dogs containing denervated, non-filtering kidneys.

METHODS AND MATERIALS

Experiments were conducted on male, mongrel dogs weighing 9-25 kg. The non-filtering kidney was prepared by the method of Blaine et al. (19). On day 1, dogs were anesthetized with sodium thiamylal (18 mg/kg i.v.) and maintained with methoxyflurane. The kidney was exposed through a flank incision using sterile technique. The ureter was then ligated and a 2.5 hour period of renal ischemia was induced by clamping the renal artery. Following the ischemic period, the clamp was removed, the incision closed and the animal allowed to recover.

Experiments were performed four days following the initial surgery. Prior to experimentation, dogs were anesthetized with sodium pentobarbitol (30 mg/kg i.v.). Following insertion of a cuffed endotracheal tube, the animals were artificially ventillated (Harvard Apparatus Co.). A polyethylene catheter was inserted into a femoral artery and positioned in the abdominal aorta for recording of the arterial blood pressure and collection of arterial blood samples. Blood pressure was recorded using a strain guage pressure transducer (Statham P23 Db) and a direct writing oscillograph (Grass Polygraph). Catheters were positioned in the abdominal vena cava via both femoral veins for injection of furosemide, infusion of d or d,l-propranolol and for administration of maintenance doses of anesthetic. In all experiments, renal blood flow was obtained by placing non-cannulating electromagnetic flowmeter probes on the renal arteries (Carolina

Medical Electronics Inc.) and recording flow simultaneously on the oscillograph. Renal venous blood samples were collected by placing a curved 20 guage needle attached to polyethylene tubing into the renal vein. All samples were collected in a volume of 2 milliliters for the determination of plasma renin concentration. Any animal whose blood pressure decreased more than 20 mm Hg from control values throughout the experiment was excluded. At the end of each experiment, a polyethylene catheter was placed in the ureter of the non-filtering kidney and the clearance of inulin was observed in two clearance periods of ten minutes each. A non-filtering kidney producing a GFR greater than 3 ml/min was rejected.

Experiment A: Effect of d-Propranolol on Furosemide Induced Renin Secretion in the Filtering and Non-Filtering Kidney.

In 6 dogs, both the filtering and non-filtering kidney were exposed via a midline incision. A one hour recovery period was allowed following surgery on each animal. Following surgical recovery, two control arterial and renal venous samples were obtained at ten minute intervals. D-propranolol was then injected in a bolus (1 mg/kg i.v.) and followed by a continuous infusion of 1 mg/kg/hr. Twenty-five minutes were allowed for equilibration and two renal venous and arterial samples were again collected at 10 minute intervals. Furosemide was then injected (5 mg/kg i.v.). Following a 10 minute equilibration period, two blood samples were obtained at 10 minute intervals.

Experiment B: Effect of Lidocaine on Furosemide Induced Renin Secretion in the Non-Filtering Kidney.

In 6 dogs, the effect of lidocaine on furosemide induced renin secretion was determined. The retroperitoneal flank incision was reopened and the non-filtering kidney exposed. The renal artery and vein were then isolated. A curved 22 guage needle attached to polyethylene tubing was placed in the renal artery, distal to the flow probe for the intrarenal infusion of lidocaine. Following the collection of two control arterial and renal venous blood samples, lidocaine was infused intrarenally for 25 minutes at a rate of 2 mg/kg/hr and then the infusion rate was reduced to 1 mg/kg/hr. Following the initial 25 minute infusion period, two blood samples were collected at 10 minute intervals. Furosemide was injected (5 mg/kg i.v.) and again following a 10 minute equilibration period, blood samples were obtained.

Experiment C: Effect of Renal Denervation on Furosemide Induced Renin Secretion in the Non-Filtering Kidney.

Denervated, non-filtering kidneys were produced in 6 male mongrel dogs (14-23 kg) by relocating the left kidney of each animal into the neck. Approximately 10 cm of the left jugular vein and left common carotid artery were isolated in the neck of the animal. During the 2.5 hour ischemic interval, utilized in the production of a non-filtering kidney, the kidney was removed from the flank with approximately 4 cm of renal artery and renal vein remaining intact with the kidney. The carotid and jugular were then ligated distally and clamped

with cardiovascular clamps proximally. The artery and vein were severed near the distal ligation. Anastomoses were then made between the carotid artery and renal artery and the jugular vein and renal vein using 6-O Mersilene cardiovascular suture (Ethicon Inc.). At all times the kidney was kept moist with sterile, isotonic saline. A pouch was formed for the kidney by surgically separating the epidermis from its connective tissue. Following completion of the 2.5 hour ischemic period, the clamps were removed and blood flow returned to the kidney. Any bleeding from the anastomoses was allowed to clot completely before closing the wound. The kidney was placed in the pouch and both the neck and flank incisions were closed and the animal allowed to recover.

On day 3, a bilateral adrenalectomy was performed via a midline incision, again utilizing sterile technique. At this time, the intact filtering kidney was also removed. Glucocorticoids were replaced with 50 mg injections of prednisolone sodium succinate (Solu-Delta-Cortef, The Upjohn Co.) both following adrenalectomy and prior to anesthetizing the animal for experimentation.

On day 4, experiments were conducted on the denervated, non-filtering kidney. The neck incision was reopened and the renal artery and renal vein were isolated. Control blood samples were collected and furosemide was then injected (5 mg/kg i.v.). Arterial and renal venous samples were collected at 5 minutes, 10 minutes and 20 minutes following furosemide injection.

Experiment D: Effect of d or d,l-Propranolol on Furosemide Induced Renin Secretion in the Denervated, Non-Filtering Kidney.

In 7 dogs, denervated, non-filtering kidneys were again produced and prepared for experimentation as described in Experiment C. Adrenalectomies were also performed on these animals 3 days following the initial surgery. Following the collection of control samples, propranolol was injected in a priming dose (1 mg/kg i.v.) and maintained with a 1 mg/kg/hr infusion. Three dogs received the d-isomer of propranolol and four dogs received the d,l-isomer. Twenty-five minutes again were allowed for equilibration and two blood samples were obtained at 10 minute intervals. Furosemide was then injected (5 mg/kg i.v.) and blood samples were once more collected at 10 minute intervals.

Analytical and Statistical Procedures

In all arterial blood samples, hematocrit was determined using the micro-method. Arterial and renal venous plasma samples and urine samples were analyzed for inulin concentration using the diphenylamine method described by Walser et al. (154). Renin activity of arterial and renal venous plasma was determined by radioimmunoassay for the generated angiotensin I (55).

Blood pressure and renal blood flow were determined directly from the oscillograph recordings and the renal resistance was computed as the quotient of the systemic blood pressure and renal blood flow. Renal plasma flow was calculated from the renal blood flow and hematocrit. Renin secretion was calculated as the product of the renal plasma flow and the renal

venous-arterial renin activity difference and was expressed as nanograms secreted per minute.

Results of experiments A, B and D were analyzed by a two-way analysis of variance and mean differences were determined by the Student-Neuman Keuls test (130). Log plots were utilized to express renin secretion since significant heterogeniety of variance existed between animals. The design of Experiment C allowed analysis by a paired t-test. The 0.05 level of probability was used as the criterion of significance.

RESULTS

Experiment A: Effect of d-Propranolol on Furosemide Induced Renin Secretion in the Filtering and Non-Filtering Kidney.

In these 6 dogs, treatment with d-propranolol decreased renin secretion in the filtering kidney from a mean control value of 1687 ± 259 ng/min to 912 ± 372 ng/min (figure I) while renal resistance was increased (table I). Subsequent administration of furosemide increased renin secretion to 1845 ± 495 ng/min (figure I) and renal resistance remained unchanged (table I).

In the non-filtering kidney, d-propranolol again decreased renin secretion from 430 ± 84 ng/min to 118 ± 39 ng/min (Figure I) and increased renal resistance (Table I). Following d-propranolol, treatment with furosemide produced a renin secretion value of 204 ± 64 ng/min which was not significantly different from the d-propranolol period (Figure I). Renal resistance remained unchanged (Table I).

Experiment B: Effect of Lidocaine on Furosemide Induced Renin Secretion in the Non-Filtering Kidney.

Treatment of the non-filtering kidney with lidocaine produced a highly variable response in renin secretion rates. However, after lidocaine, the administration of furosemide produced no further change in renin secretion in five out of six dogs (Figure II). There was no significant change in renal resistance following lidocaine or furosemide treatment (Table I).

FIGURE I

Effect of d-propranolol on the basal rate of renin secretion and furosemide induced renin secretion in the filtering and non-filtering kidney. Mean ± sem of 7 experiments are shown. C is the control rate of renin secretion. D-propranolol (d-P) produced a significant decrease ($p<0.05$) in renin secretion in both the filtering and non-filtering kidney. Furosemide (F) produced a significant increase ($p<0.05$) in renin secretion in the filtering kidney.

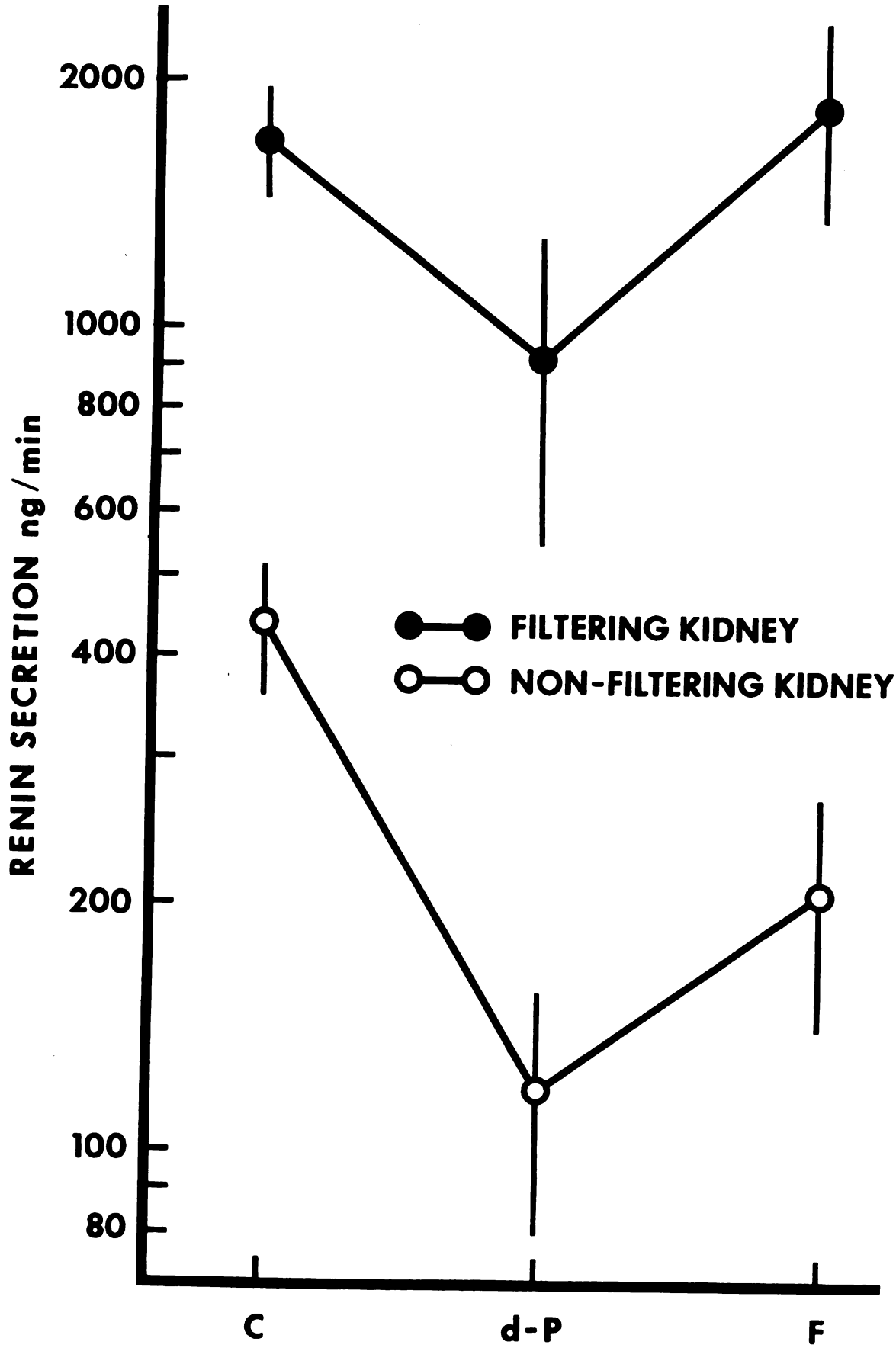

2000
1000
800
600
400
200
100
80
RENIN SECRETION ng/min
FILTERING KIDNEY
NON-FILTERING KIDNEY
C
d-P
F

FIGURE II

Effect of lidocaine on the basal rate of renin secretion and furosemide induced renin secretion in the non-filtering kidney. Renin secretion rates of 6 experiments are shown.

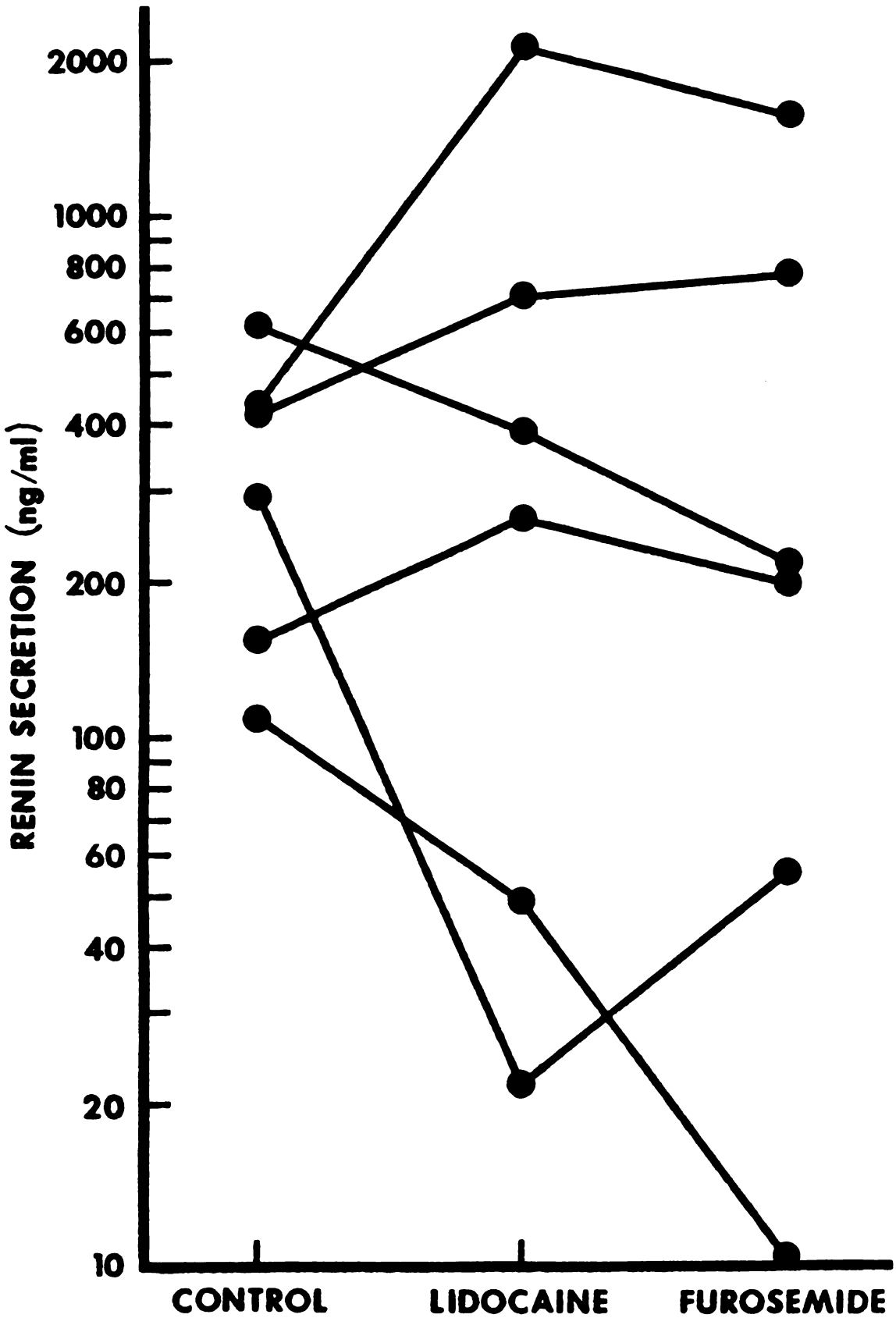

2000
1000
800
600
400
200
100
80
60
40
20
10
RENIN SECRETION (ng/ml)
CONTROL
LIDOCAINE
FUROSEMIDE

TABLE I

Effect of treatment with Propranolol (P), Lidocaine (L) and Furosemide (F) on Renal Resistance in the Filtering Kidney (FK), Non-Filtering Kidney (NFK) and Denervated Non-Filtering Kidney (D-NFK).

Experiment	Renal Resistance (pru)		
	C	d-P	F
FK (6)	1.04 ± 0.17	1.39 ± 0.28*	1.28 ± 0.22
NFK (6)	1.76 ± 0.37	2.48 ± 0.57*	3.11 ± 0.78
	C	L	F
NFK (6)	1.80 ± 0.41	2.38 ± 0.88	2.77 ± 1.13
	C		F
D-NFK (6)	1.11 ± 0.19		0.95 ± 0.16*
	C	d or d,1-P	F
D-NFK (7)	2.10 ± 0.34	2.16 ± 0.34	2.15 ± 0.49

C = Control; pru = peripheral resistance units; ( ) = n; *p < 0.05.

Experiment C: Effect of Renal Denervation on Furosemide Induced Renin Secretion in the Non-Filtering Kidney.

In 6 dogs with denervated, non-filtering kidneys, furosemide increased renin secretion from control values averaging 117 ± 80 ng/min to 731 ± 273 ng/min (Figure III). This rise in renin secretion was accompanied by a significant decrease in renal resistance, from a mean control value of 1.11 ± 0.19 pru to 0.95 ± 0.16 pru (Table I).

Experiment D: Effect of d or d,l-Propranolol on Furosemide Induced Renin Secretion in the Denervated, Non-Filtering Kidney.

In 7 animals with denervated, non-filtering kidneys, the effect of d or d,l-propranolol was studied on furosemide induced renin secretion. In four out of four dogs, d,l-propranolol decreased renin secretion while d-propranolol decreased renin secretion in two out of three dogs (Figure IV). D or d,l-propranolol produced no change in renal resistance from a control value of 2.10 ± 0.34 pru to 2.16 ± 0.34 pru (Table I). Treatment with furosemide decreased or did not significantly change renin secretion in all seven animals (Figure IV). Furosemide did not produce a change in renal resistance from a value of 2.16 ± 0.34 pru in the propranolol period to 2.15 ± 0.49 pru (Table I).

FIGURE III

Effect of furosemide on renin secretion in the denervated, non-filtering kidney. Mean ± sem of 6 experiments are shown. Furosemide produced a significant increase in renin secretion ($p<0.05$).

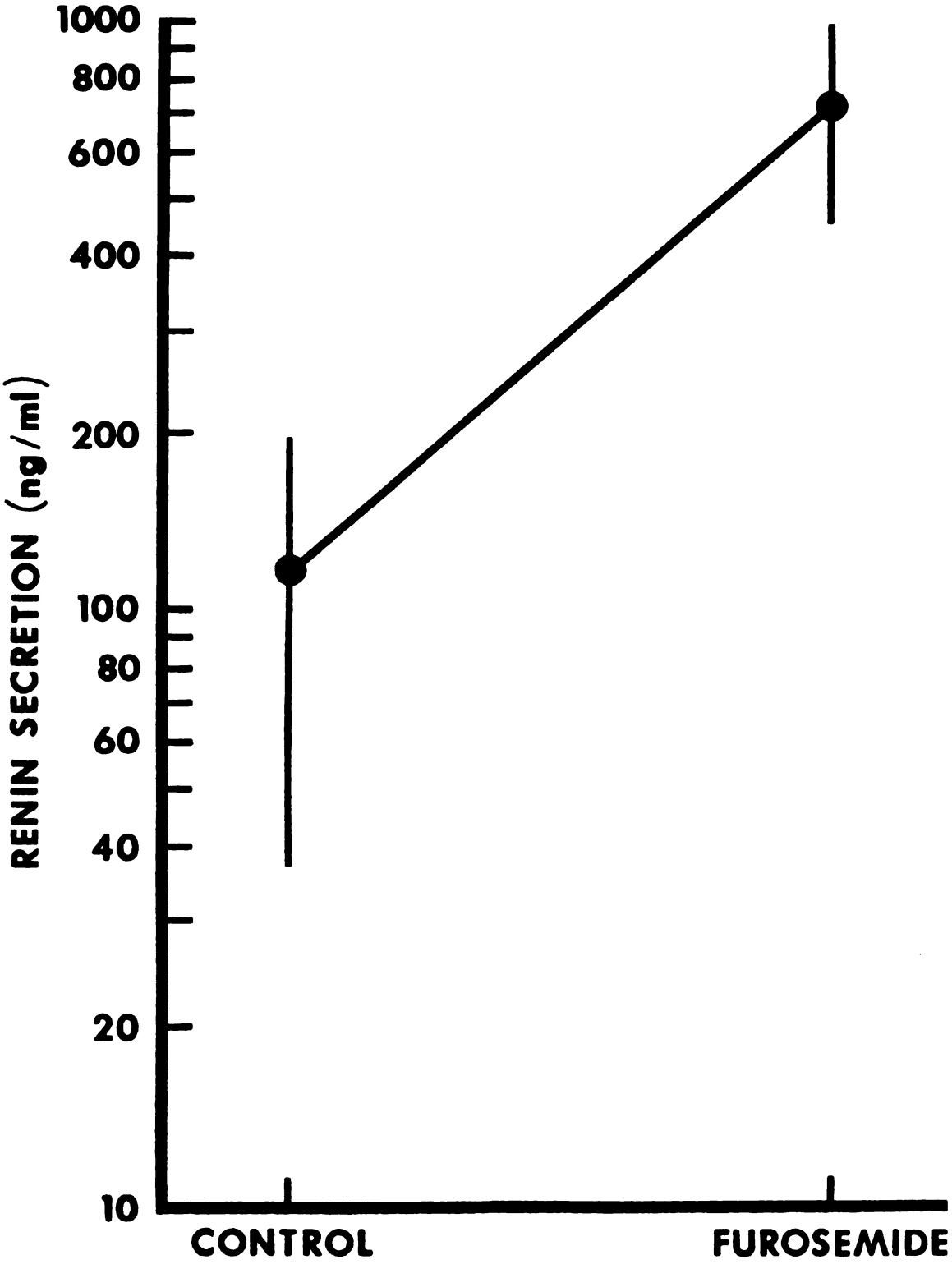
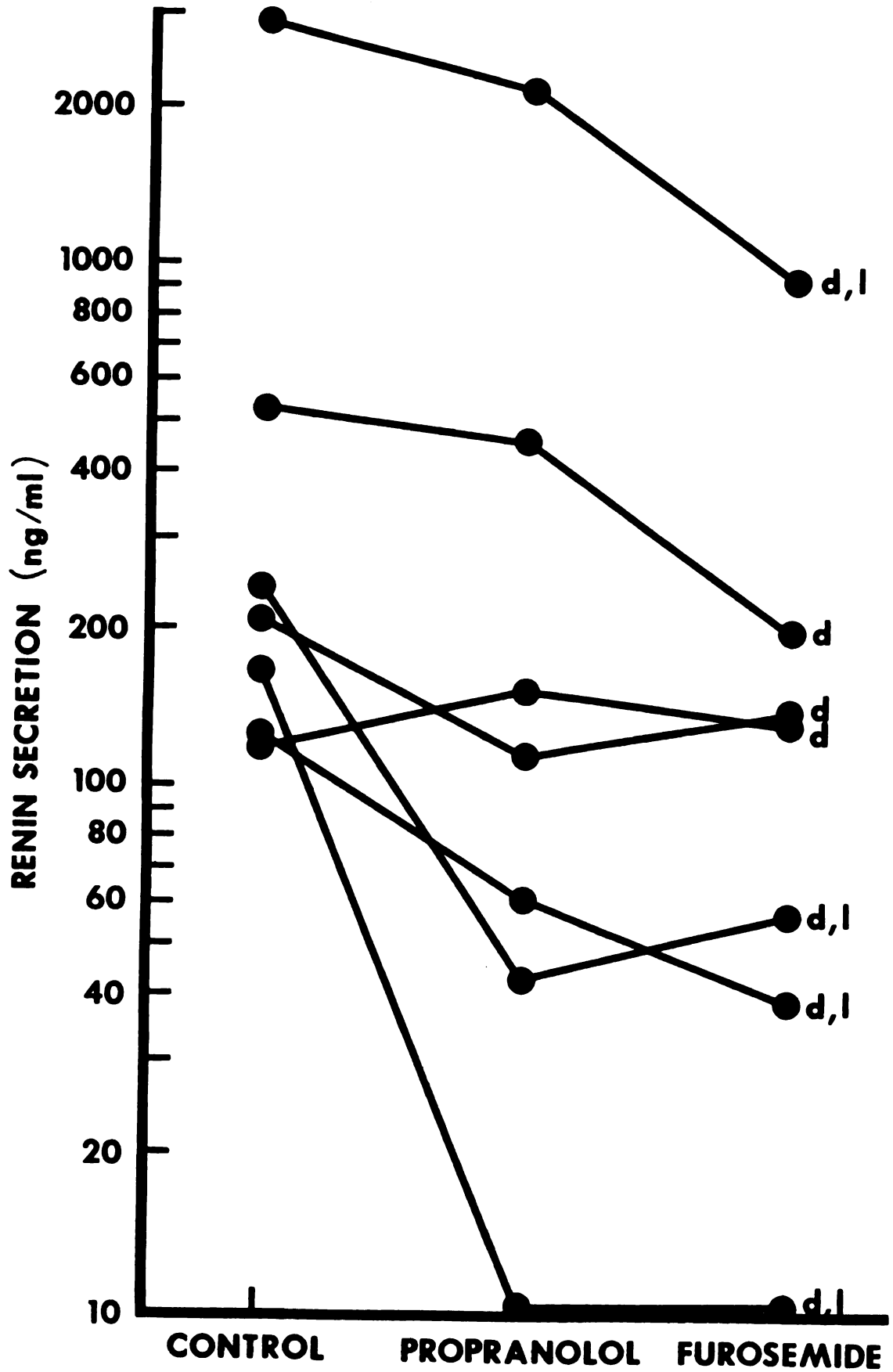

1000
800
600
400
200
100
80
60
40
20
10
RENIN SECRETION (ng/ml)
CONTROL
FUROSEMIDE

FIGURE IV

Effect of d or d,l-propranolol on the basal rate of renin secretion and furosemide induced renin secretion in the denervated, non-filtering kidney. Renin secretion rates of 7 experiments are shown.


RENIN SECRETION (ng/ml)
2000
1000
800
600
400
200
100
80
60
40
20
10
d,l
d
d
d
d,l
d,l
d,l
CONTROL
PROPRANOLOL
FUROSEMIDE

DISCUSSION

The control of renin secretion on the vascular side of the juxtaglomerular apparatus (JGA) appears to occur by two mechanisms. The baroreceptor within the afferent glomerular arteriole may respond to changes in the vascular transmural pressure gradient or arteriolar wall tension (19, 127, 128). Alterations in the stimuli of the sympathetic nervous system also change renin secretion, however, the exact mechanism of action is not clear.

In order to effectively study these mechanisms, differentiation of the macula densa receptor, baroreceptor and sympathetic nervous system must be accomplished. Blaine and coworkers have separated the tubular and vascular effects by the use of the non-filtering kidney model (19). In the non-filtering kidney, sodium delivery on the tubular side of the macula densa is eliminated and sodium delivery on the vascular side is held constant. Thus, changes in glomerular filtration rate or filtered sodium cannot produce any changes in renin secretion.

Blockade of glomerular filtration in the non-filtering kidney was demonstrated by Blaine et al. (19), by observation for lissamine green dye in the surface tubules following dye injection into the renal artery. In some of the present experiments, these observations were confirmed. In addition, at the end of each experiment the clearance of inulin was found to be less than 3 ml/min in all non-filtering kidneys.

In the intact non-filtering kidney, renin secretion may still be altered on the vascular side of the juxtaglomerular apparatus by the baroreceptor mechanism (37, 19, 18, 51, 17, 162, 161, 32), the sympathetic nervous system (161, 70) or by an action of the sympathetic nerves on the baroreceptor. By transplanting a non-filtering kidney, denervation was accomplished in a kidney with the vascular receptor effectively isolated from the macula densa receptor.

Adrenalectomies were performed in experiments involving denervated non-filtering kidneys to eliminate the primary endogenous source of catecholamine. Denervation of the non-filtering kidney was verified by the fluorometric determination of norepinephrine levels (31) in three intact kidneys and three transplanted kidneys. Norepinephrine concentration in the intact kidney ranged from 0.04 μg per gram of tissue to 0.25 μg/g. No norepinephrine was detectable in the denervated, non-filtering kidneys.

D,l-propranolol contains both the property of beta adrenergic blockade which is specific to the l-isomer and membrane stabilizing properties similar to local anesthetics, which are specific to both isomers (13, 43). It was demonstrated by Barrett and Cullum (13) that d and l-propranolol effectively inhibited the aortic strip contraction induced by electrical stimulation. Similarly, these isomers blocked the action potential response to electrical stimulation of the frog sciatic nerve.

Recently, propranolol was shown to decrease renin secretion

in the denervated, non-filtering kidney as evidence for an intrarenal beta adrenergic mechanism mediating renin release (69). The beta adrenergic agonist, isoproterenol, has been shown to effectively stimulate renin secretion (7, 71, 82, 149). This stimulation has been attributed to these beta agonist properties since the renin response was unaltered in the presence of phenoxybenzamine (7, 149). D,l-propranolol was shown to block the isoproterenol induced renin secretion (7, 149). In this same experiment, d-propranolol had no effect on the renin response to isoproterenol (7). In the present study, d-propranolol did not affect the decrease in blood pressure or increase in heart rate produced by intravenous, isoproterenol injection. However, beta adrenergic blockade by d,l-propranolol effectively inhibited these isoproterenol induced responses (Appendix I).

It has been demonstrated that d,l-propranolol decreases renin secretion in the filtering kidney (37, 67) as well as in the non-filtering kidney (37). Similarly, d,l-propranolol blocks renal nerve stimulated increases in renin secretion (67, 36, 68) and this blockade has been attributed to the l-isomer <u>in vitro</u> (104). Furthermore, d-propranolol did not block the basal rate of renin release in the rabbit (156) and failed to block renin release to a variety of adrenergic stimuli (104, 156, 68). Thus, the action of d,l-propranolol on plasma renin activity has previously been attributed to the beta blocking activity of the l-isomer.

The diuretic furosemide, has been shown to stimulate

renin secretion at both the vascular (37) and macula densa receptors (93, 9, 145). The activity of this vascular receptor has been attributed to the baroreceptor mechanism. Supra-renal aortic constriction (19, 51, 18, 162) and the infusion of vasodilating agents such as papaverine (161), minoxodil (112), hydralazine (112) or furosemide (37, 93, 145, 9) produce increases in renin release accompanied by decreasing renal resistance. Corsini et al. (36), demonstrated that the vasodilating action of furosemide will produce an increase in renin secretion in the non-filtering kidney.

In the present experiments, d-propranolol significantly decreased renin secretion in both the filtering and non-filtering kidney (Figure I) while renal resistance increased in both kidneys (Table I). Furosemide produced a significant rise in renin secretion (Figure I) and did not alter renal resistance (Table I) following baroreceptor blockade by d-propranolol in the filtering kidney. This increase is attributed to alterations in sodium delivery to the functional macula densa mechanism. In the non-filtering kidney, however, furosemide failed to produce a significant increase in renin secretion following treatment with d-propranolol (Figure I). This inability of furosemide to stimulate renin secretion following d-propranolol in the non-filtering kidney is associated with no further change in renal resistance (Table I).

In order to confirm that the drug being used was purely the d-isomer, an optical rotation study was performed. The d-propranolol produced a specific rotation $[\alpha]_D^{21}$ of +25.3

degrees while the specific rotation of the racemic mixture was 0.0 degrees. The specific rotation $[\alpha]_D^{21}$ of d-propranolol has been previously reported as +22.2 degrees (13).

These data indicate that the membrane stabilizing property of d-propranolol provides effective blockade of the baroreceptor on the vascular side of the juxtaglomerular apparatus resulting in a decrease in renin secretion in both the filtering and non-filtering kidney. These results suggest that the ability of d,l-propranolol to decrease renin secretion may be partially due to the membrane stabilizing activity of the d-isomer.

These data were further substantiated in Experiment B. Lidocaine administration produced a highly variable response on basal rates of renin secretion (Figure II). However, in these animals when renal resistance decreased, renin secretion increased. Conversely, in kidneys where renal resistance increased, a correlation of decreased renin secretion could be made. Following baroreceptor blockade by lidocaine, furosemide again failed to increase renin secretion in five out of six animals (Figure II). Renal resistance was also unchanged (Table I).

In both Experiments A and B, furosemide did not alter renal resistance following baroreceptor blockade (Table I). Since furosemide does not produce a similar increase in renin secretion in the d-propranolol or lidocaine treated non-filtering kidney, the vascular and macula densa receptors must be acting in an independent manner in their ability to alter renin

release.

Several studies have demonstrated that the sympathetic nervous system may stimulate renin release by a direct action on the JGA (11, 70, 6, 94). However, this innervation may be exerting an affect on the vascular receptor in the afferent arteriole. This modulation of the vascular receptor may produce changes in the vascular tone of the afferent arteriolar smooth muscle or changes in glomerular filtration rate.

Renal denervation has been reported to abolish the renin response of furosemide in the dog (101). It was suggested by these authors that neurogenic tone provided a threshold for the direct action of furosemide on the vascular side of the JGA. In the present study, treatment of the denervated, non-filtering kidney with furosemide produced an increase in renin secretion (Figure III) and a decrease in renal resistance (Table I). This result demonstrates that furosemide induced renin secretion at the vascular site is not dependent on the sympathetic nervous system.

In Experiment D, administration of d or d,l-propranolol decreased renin secretion in six out of seven dogs (Figure IV). Following propranolol, furosemide failed to alter renal resistance (Table I) and renin secretion (Figure IV) in all seven animals. These data further substantiate that the ability of d,l-propranolol to decrease renin secretion may be due to the membrane stabalizing activity of the d-isomer on the vascular receptor.

SUMMARY AND CONCLUSIONS

The results presented, indicate that the ability of d,l-propranolol to decrease renin secretion may be partially attributed to the membrane stabilizing activity of the d-isomer. Substantiation is also made for the presence of two separate receptor sites participating in the control of renin secretion. The stimulation of renin secretion by vasodilatation with furosemide is not dependant upon renal innervation. It has been demonstrated that the actions of the vascular receptor, sympathetic nervous system and macula densa receptor may alter renin secretion in an independent manner.

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APPENDIX I

Effect of d and d,l-propranolol on isoproterenol induced changes in heart rate and blood pressure from control. Heart rate is expressed as the change in beats/min from control. Blood pressure is expressed as the change in mm Hg from control.

HEART RATE (Δ beats/min)

Isoprot dose µg/kg	Isoprot	d-P + Isoprot	d,l-P + Isoprot
0.1	+8.0	+13.0	0.0
0.2	+18.0	+19.5	0.0
	+37.0	+18.0	0.0
0.3	+24.0	+28.0	0.0

BLOOD PRESSURE (Δ mm Hg)

Isoprot dose µg/kg	Isoprot	d-P + Isoprot	d,l-P + Isoprot
0.1	-18.0	-5.3	-
	-10.0	-8.0	-2.3
0.2	-16.0	-14.0	-
	-34.0	-29.0	0.0
	-23.0	-27.0	-2.0
0.3	-55.0	-35.0	-
	-16.0	-14.0	-12.0
0.5	-31.0	-15.0	-
	-	-35.0	-

Isoprot = Isoproterenol
d-P = d-Propranolol
d,l-P = d,l-Propranolol