HORMONAL INFLUENCE ON RENAL HEMODYNAMICS: RELATION OF PROSTAGLANDIN E_2 AND THE RENIN-ANGIOTENSIN SYSTEM TO FUROSEMIDE INDUCED CHANGES IN RENAL BLOOD FLOW

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
John T. Orley
1976

ABSTRACT

HORMONAL INFLUENCE ON RENAL HYMODYNAMICS:
RELATION OF PROSTAGLANDIN E_2 AND THE RENIN-ANGIOTENSIN
SYSTEM TO FUROSEMIDE INDUCED CHANGES
IN RENAL BLOOD FLOW

By

John T. Orley

Due to its actions to increase renal blood flow and renin secretion, the potent diuretic, furosemide, was used in these experiments as a tool to further evaluate the influence of renal prostaglandins and the renin-angiotensin system on renal hemodynamics. In order to isolate the effects of the two hormonal systems on each other and on renal blood flow, prostaglandin synthetase and renin-angiotensin inhibitors were used prior to giving furosemide to volume expanded dogs.

Renal blood flow, prostaglandin E_2 (PGE_2) secretion, renin secretion, and sodium excretion were the primary parameters determined in dogs during hydropenia, volume expansion with isotonic saline, volume expansion plus treatment, and volume expansion plus furosemide infusion into the renal artery (0.015 mg/min/kg). Treatment included indomethacin (3.5 mg/kg) and SQ 20,881 (1.5 mg/kg). In addition, 1-sarcosine-8-alanine angiotensin II (saralasin) was infused

into the renal artery (0.5 μg/kg/min) of volume expanded animals both prior to and in combination with furosemide. Renal blood flow was determined using an electromagnetic flowmeter and PGE_2 in plasma was determined by radioimmunoassay. Following volume expansion, renin secretion was decreased while PGE_2 secretion was unchanged. In volume expanded animals, furosemide produced a significant increase in renal blood flow and PGE_2 secretion while in animals not subjected to volume expansion no significant increase in either parameter was observed. Indomethacin inhibited PGE_2 secretion reducing the ability of furosemide to cause an increase in renal blood flow. Furosemide did not significantly increase PGE_2 secretion and its effect on renal blood flow was blunted in animals pretreated with SQ 20,881. Neither SQ 20,881 nor indomethacin were found to alter renin secretion or sodium excretion prior to or after furosemide infusion. The effect of furosemide to increase renal blood flow and PGE_2 secretion was not significantly altered in animals treated with saralasin.

These data suggest that the furosemide induced increase in renal blood flow is in part mediated by renal prostaglandins and potentiated under volume expanded conditions. Furthermore, constituents of the renin-angiotensin system demonstrate minimal influence on renal blood flow and

prostaglandin secretion either prior to or after furosemide. It is also shown that the natriuretic and diuretic effects of furosemide are unrelated to the release of prostaglandins.

HORMONAL INFLUENCE ON RENAL HEMODYNAMICS: RELATION OF PROSTAGLANDIN E_2 AND THE RENIN-ANGIOTENSIN SYSTEM TO FUROSEMIDE INDUCED CHANGES IN RENAL BLOOD FLOW

By

John T. Orley

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1976

ACKNOWLEDGEMENTS

The author wishes to express his thanks and appreciation to Dr. M. D. Bailie for his interest, and invaluable instruction during the course of this graduate program. He originated the idea for this study, and his confidence and direction have made this study an enjoyable and enriching experience.

Special thanks are due to Dr. J. B. Hook for providing invaluable advice during the course of this study and for the many articles from the literature which he has provided.

I would also like to thank my lovely wife-to-be, Dianne, for her understanding and encouragement which enabled me to complete this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES--continued

FIGURE Page

INTRODUCTION

The mechanism involved in the autonomous and intrinsic regulation of renal circulation has been an elusive and controversial subject ever since this phenomenon was first realized. With the discovery of the intrarenal renin-angiotensin system a mechanism under hormonal influence was suggested. Thurau presented the renin-angiotensin system as the integral factor involved in the regulation of renal circulation (95). Studies which imposed alterations on the renin-angiotensin system produced a variety of effects on the autoregulatory response, many of which gave no support to Thurau's proposal (9,23,32,60,85). Recent observations have demonstrated that another hormonal system, located in the kidney, is composed of prostaglandins and exhibits vasoactivity (58,59). The primary action upon infusion of prostaglandins into the kidney is to cause vasodilation and increased renal blood flow (97). Furthermore, agents inhibiting the synthesis of prostaglandins decrease resting renal blood flow and increase renal resistance (61,80). A relationship between the two systems was proposed by Aiken and Vane who observed an increased release of a PGE-like material from the kidney upon the infusion of angiotensin II into the renal artery (1). Since the two systems seem to

influence each other and display vasoactive properties antagonistic toward one another, investigators have suggested that the interaction between the two systems may be an important factor in the regulation of renal circulation (45,73).

The potent diuretic furosemide has been shown to cause an increase in renal blood flow (8,106) and renin secretion (98). It has also been suggested that the diuretic increases prostaglandin secretion (8,106). Because of these actions, furosemide was used in this study as a tool to further evaluate the influence of the renin-angiotensin and prostaglandin systems on each other and on changes in renal blood flow. Inhibitors of prostaglandin synthesis and the renin-angiotensin system were also used to further evaluate the question of the involvement of prostaglandins and the renin-angiotensin system in the changes in renal blood flow induced by furosemide.

HISTORICAL REVIEW

Early History

A biologically active constituent in human semen was first indicated in the studies of Kurzrok and Lieb in 1930 in which the action of human seminal fluid on isolated uterus strips was observed (56). Following considerable advancement in pharmacological analysis of biologically occurring active substances, Goldblatt and von Euler differentiated the actions of human seminal extracts on isolated organs from the then known generally occurring compounds (21,29). Since the active compound in these extracts appeared to be unknown, von Euler named them prostaglandins, owing to their appearance in extracts of the prostate and vesicular gland. It was not until Bergstrom and colleagues (12) had isolated crystalin prostaglandin F (PGF) in 1957 and prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) and E_1 (PGE_1) in 1960 that the enthusiasm for investigation of these molecules in the fields of biochemistry, physiology and pharmacology intensified. A total of 13 different prostaglandins were revealed following the initial isolations. Differentiations within the family of prostaglandins were synthesized from the parent substance, prostanoic acid.

The biosynthesis of prostaglandins was first accomplished by Van Dorp and Bergstrom independently when they produced prostaglandin E_2 (PGE_2) from the enzymatic conversion of arachidonic acid (11,99). The metabolism of these substances was first elucidated when Hamberg and Samuelsson identified one urinary metabolite of PGE_2 in man (35). Their scheme of degredation of PGE_2 which resulted in the metabolite 7_α-hydroxy-5,11-di-oxo-tetranor-prosta-1, 16-dioic acid was as follows: oxidation of C-15 by prostaglandin-15-hydroxy dehydrogenase in the lungs, liver, and kidney, reduction of the C-13,14 double bond by prostaglandin reductase followed by β and ω oxidation (43).

As advances in scientific methodology continued, so too did the understanding of the biochemistry and physiological actions of the newly discovered prostaglandins. The first method used to identify prostaglandins was developed by Anggard in 1965 (4). In this test the unknown was considered a prostaglandin if its biological activity was greatly reduced by incubation with a preparation of the enzyme prostaglandin 15-hydroxy dehydrogenase, the major metabolizing enzyme specific for prostaglandins. Other inactivation agents were used to separate individual prostaglandins from a conglomeration of many. The importance of the biological assay in relation to the quantitative analysis of prostaglandins was realized when these substances

were shown to have differential actions on blood pressure and various isolated intestinal and uterine smooth muscle preparations. Prostaglandins of the A, E, and F series could be distinguished readily by parallel assay on appropriate tissues (58,59). Investigators have shown the quantitative parallel assay to be a powerful tool, particularly if results were obtained on three or more tissues (15,62,70). An extension of the parallel assay was developed by Vane (101). In this model the blood or perfusion fluid from an animal or organ is allowed to drip over the surface of smooth muscle preparations selected for their sensitivity to the endogenous prostaglandins. With this method, information concerning the release and disappearance of biologically active compounds could be readily obtained. This model allowed the investigator to observe the time of maximal periods of secretion and to correlate these periods to the concurrent physiological response of the animal. Furthermore, its use would also indicate the optimal time for collection of blood for in vitro assay of prostaglandins. Attempts to separate, identify and estimate prostaglandins were further continued with the use of gas-liquid and radio chromatography and ultraviolet, infrared, and nuclear magnetic resonance spectroscopy (53,71,72). While the sensitivity and usefulness of these methods was too questionable for wide application in research, the development of such

procedures was instrumental in furthering other methodology which proved to be more applicable in the field of prostaglandins. One such method arising from earlier studies was the development of the combined mass spectrometry and gas chromatography by Thomson, Los and Horton (94). This micro method allowed for the identification and estimation of prostaglandins in the nanogram range and has been proven to be an invaluable tool in this field.

Radioimmunoassay and competitive protein binding procedures have proven to be useful in other areas of research. Development with respect to the prostaglandins, however, must still be considered in the early stages. As of late, considerable effort is being directed toward this assay method which when validated would allow sensitive and specific quantitation of prostaglandins (15,30,38). While some success has been obtained in measuring prostaglandins E, A, and F, most radioimmunoassay methods for prostaglandin measurement are still subject to confirmation by combined gas chromatography and mass spectrometry (47,48).

History of Renal Prostaglandins

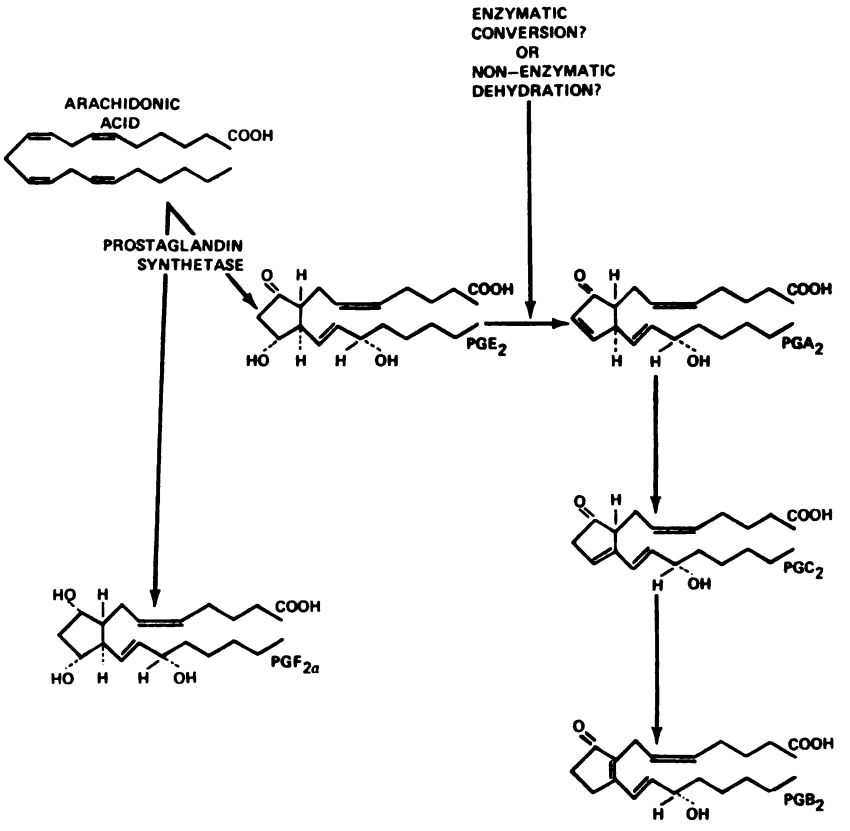
Interest in renal prostaglandins was initiated in 1965 with the discovery by Lee et al. (59), of three prostaglandin-like acidic lipids present in extracts of the medulla of the rabbit kidney, one of which was named medullin. Thin-layer chromatography, spectroscopic and

mass-spectral analysis of the acidic lipids established their structures to be identical to PGE_2, PGF_2, and PGA_2 (58). Later evidence determined that part or all of PGA_2 was formed during the isolation procedures from endogenous PGE_2 (58). PGE_2 was designated as the principle vasodepressor lipid of the rabbit renal medulla in a study conducted by Daniels (18).

Biochemistry

An enzyme system in the renal medulla of the rabbit was discovered when Hamberg (34) demonstrated the formation of PGE_2 and $PGF_{2\alpha}$ from arachidonic acid by homogenates of rabbit renomedulla tissue. A radioactive portion consisting of a large fraction of PGE_2 and a lower yield of $PGF_{2\alpha}$ was recovered from tnese homogenates following their incubation with tritiated arachidonic acid. Only traces of a labeled compound tentatively identified as PGA_2 could be isolated after incubation. In a recent review (67), McGiff suggested a scheme for the synthesis of renal prostaglandins (see the chart on the following page).

Arachidonic acid has been shown to be the only substrate for prostaglandin synthesis in the kidney (67). This substrate is converted to either PGE_2 or $PGF_{2\alpha}$ by the enzymatic complex, prostaglandin synthetase. PGE_2 is then converted to PGA_2, C_2, or B_2 by either enzymatic conversion or non-enzymatic dehydration (67). Until recently supportive


ENZYMATIC
CONVERSION?
OR
NON-ENZYMATIC
DEHYDRATION?
ARACHIDONIC
ACID
COOH
PROSTAGLANDIN
SYNTHETASE
O
H
COOH
HO
H
H
OH
PGE2
O
H
COOH
H
H
OH
PGA2
O
H
COOH
H
OH
PGC2
HO
H
COOH
HO
H
H
OH
PGF2α
O
COOH
H
OH
PGB2

evidence for the endogenous synthesis of renal PGA_2 has been lacking (17,34). However, due to newer methods of isolation and identification, a small amount of evidence suggests that PGA_2 may not be solely an artifact of PGE_2 *in vitro* dehydration but may actually be synthesized *in vivo* by enzymatic dehydration of PGE_2 (7,107).

The location of renal prostaglandin synthesis and metabolism was examined in the rabbit kidney by Larsson and Anggard (57). Their interest was focused on the biosynthesis and metabolism of prostaglandins in the cortex and the inner and outer medullary regions. They found PGE_2 formation to be highest in the inner medulla, but contrary to the results of earlier studies (16), significant biosynthesis also occurred in the cortex. While this recovery of a PGE-like material in cortical homogenates from incubation with arachidonic acid was only 10% that of the renal medulla, the importance of this level of cortical biosynthetic activity is considerable in view of the magnitude of prostaglandin biosynthetic activity of the renal medulla, surpassed only by that of seminal vesicles (67).

Metabolism of prostaglandins by prostaglandin 15-hydroxy dehydrogenase was shown to occur primarily in the cortex with levels ten times those seen in the inner medulla (57). Similar findings were reported in studies conducted in the swine kidney in which the concentration of

prostaglandin 15-hydroxy dehydrogenase was found to be three times greater in the cortex than the medulla (5). Thus, a clear-cut dissociation between sites of biosynthesis and catabolism was demonstrated in the kidney. It might be suggested, therefore, that the function of cortical prostaglandin 15-hydroxy dehydrogenase is either to inactivate prostaglandins formed in the medulla, or to protect medullary prostaglandin receptors from high levels of circulating prostaglandins.

The subcellular location of prostaglandins, prostaglandin precursor acids, prostaglandin synthetase, and prostaglandin 15-hydroxy dehydrogenase was investigated by Anggard et al. (3), in homogenates of rabbit renal papilla. It was demonstrated that PGE_2 was formed mainly from locally available arachidonate in membranes which may have been derived from either cell membranes or membranes of endoplasmic reticulum. From these observations it was proposed that following their synthesis, prostaglandins are released into the cytoplasm of the cells rather than being concentrated within specific subcellular particles. Since no detectable prostaglandin 15-hydroxy dehydrogenase was found in the supernant fraction of the papilla, it was suggested that after their release, renal prostaglandins leave the papilla without undergoing metabolic inactivation (3). An in vitro experiment by Crowshaw revealed a very small proportion of

prostaglandins associated with microsomes, mitochondria and lipid droplets which suggest that there are no sites of storage within the cellular elements of renomedullary tissue. It may, therefore, be presumed that prostaglandins are not stored in renal cells, but are synthesized and released upon appropriate stimulation (17).

Histochemical studies have revealed a variety of cell types within the kidney that may be involved in the synthesis of prostaglandins. Janszen et al. (50), observed a high concentration of prostaglandin synthetase in the cells of the collecting tubules. Muirhead et al. (75), in their tissue culture studies, obtained evidence indicating that the renomedullary interstitial cells synthesized renomedullary prostaglandins. Further investigation of prostaglandin producing renal cell types was explored by Prezyna et al. (82), who proposed the existence of a renomedullary body as a prostaglandin synthesizing tissue. This newly recognized structure of human renomedullary interstitial cell origin was shown to have a high prostaglandin content.

With the discovery of specific inhibitors of prostaglandin biosynthesis came many explanations concerning the functions of prostaglandins in the organ from which the release occurred. Indomethacin is a specific inhibitor of prostaglandin biosynthesis in all tissues so far studied including the kidney (100). It produces a dose-dependent

inhibition of prostaglandin release from the kidney, confirming that the release of prostaglandins from the kidney is the consequence of new synthesis (87). Furthermore, a concurrent decrease in renal blood flow is seen upon the administration of indomethacin (61). Since the majority of blood flow in the kidney is localized in the cortex it was assumed that the major site of action of renal prostaglandins was the vasculature perfusing the cortex.

Realizing that the major site of prostaglandin synthesis was located in the papilla and medulla (3,34,50), studies were directed toward determining the route in which renal prostaglandins were transported from the renal papilla and medulla to the cortex. It may be assumed that one pathway is via diffusion or active transport into the ascending vasa recta. Another possibility, however, has been proposed by Frolick et al. (27), who found prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ in human urine. Their evidence suggested that prostaglandins synthesized and released in the medulla may be transported up to the cortex in the ascending limb of the loop of Henle. Another study investigated the site of entry of prostaglandins into tubular fluid using the stop flow experimental method (105). Evidence from this study indicated that the site of entry into the tubular fluid appeared to be either the loop of Henle or the distal tubule, prior to the collecting duct.

These latter points of entry would provide medullary prostaglandins access to all distal sites in the nephron, including its juxtaposition with glomerular arterioles. This access would allow medullary vasodilator prostaglandins to express a more profound action on renal blood flow than if their action were restricted to the low blood flow areas of the medulla and papilla. Entry into the tubular fluid at the ascending loop of Henle would also allow renal prostaglandins to participate in the reabsorptive and secretory processes of the kidney tubules.

While many functional implications arose from the identification of prostaglandins in human urine, a considerable advancement in the evaluation of prostaglandin biosynthesis was also determined. Stimuli which increased the synthesis and release of prostaglandins into renal venous blood also produced concurrent increases in prostaglandin concentration in the urine (28). Agents used to inhibit prostaglandin synthesis were shown to significantly decrease prostaglandin concentration in tubular fluid (27). Thus, it was proposed that urinary prostaglandins are a reflection of renal prostaglandin synthesis and could be used as a tool to delineate renal prostaglandin physiology and pathology.

As mentioned previously, the primary location for intrarenal inactivation of prostaglandins is the cortex, due to its high concentration of prostaglandin 15-hydroxy

dehydrogenase (5,57). A more specific intrarenal distribution of prostaglandin 15-hydroxy dehydrogenase activity was provided in a report presented by Nessen et al. (79). The most pronounced activity was observed in the thick ascending limb of the loop of Henle and the distal tubule. Lesser activity was found in the collecting tubules of the inner medulla and cell structures comprising the medulla. Considerable activity was also found in the tunica media of the cortical arteries and arterioles and in the visceral epithelium of the renal corpuscles. This report also noted that NAD was required as a factor for prostaglandin 15-hydroxy dehydrogenase which demonstrated high substrate specificity.

Since it can be seen that if the physiological activity of prostaglandins is located in close relation to their place of inactivation, then the location of prostaglandin 15-hydroxy dehydrogenase may indicate not only the place of inactivation but also the locale of physiological action. Localization of an intense prostaglandin 15-hydroxy dehydrogenase activity in the cortical arteries and arterioles may consequently indicate a physiological action followed by a biological inactivation taking place in these vessels for prostaglandins endogenous to the kidney. It is due to their location of synthesis, metabolism and the rate of transportation that renal prostaglandins are presently considered as

locally acting hormones. Other evidence supporting the role of prostaglandins as local hormones was presented in the findings of Terriera and Vane (22). They demonstrated that both PGE and PGF were at least 95% inactivated in cats and dogs during a single passage through the lungs. It was shown, however, that PGA and A_2 were not subject to destruction by the lung and therefore, merited consideration as possible circulating hormones (69).

Physiological Role of Prostaglandins

The physiological relevance of renal prostaglandins was first indicated in the studies of Hieber et al. (41), who demonstrated that the substance called medullin and later identified as PGA_2 causes renal vasodilation when injected into the kidneys of rats and dogs. Vander (97) observed several renal responses when low doses of PGE_1 were infused into the renal artery of anesthetized dogs. In this study, urine flow, sodium excretion, free water clearance and renal blood flow increased, while there was no change in glomerular filtration rate and PAH extraction decreased during PGE_1 infusion. There was no effect on the contralateral kidney, blood pressure, or heart rate. Similar results supportive of Vander's observations were noted when arachidonic acid and PGE_2 were infused into the renal artery of dogs (93). Indomethacin inhibited all the effects of arachidonic acid infusion while none of the

effects produced by the infusion of PGE_2 into the renal artery were inhibited. Lonigro et al. (61), observed a direct relationship between the synthesis of PGE_2 and renal blood flow after systemic injection of indomethacin. In this study, inhibition of prostaglandin synthesis in anesthetized dogs reduced renal blood flow and this reduction closely correlated with a decline in the basal concentration of a PGE-like substance in renal venous blood to 0.06 ± 0.02 ng/ml from a mean control value of 0.34 ± 0.10 ng/ml. Due to the renal actions observed upon the infusion of prostaglandins or arachidonic acid into the mammalian kidney and the effect of prostaglandin synthetase inhibitors on these actions it was proposed that endogenous renal prostaglandins have physiological importance as regulators of renal function.

Further investigation continued in an attempt to determine the interaction of prostaglandins with other factors known to influence renal function. Stimuli which produced changes in renal blood flow were employed with the expectation that their action on renal blood flow could be associated to their effect on prostaglandin synthesis in the kidney. Dunham and Zimmerman demonstrated that sympathetic nervous stimulation produced an increase in the release of a prostaglandin-like material from the dog kidney (20). Vascular constriction elicited by renal nerve stimulation

markedly enhanced a low basal efflux of the prostaglandin-like material. Similar results were obtained when they infused norepinephrine into the renal artery. Another study demonstrated that norepinephrine and epinephrine while causing vasoconstriction also brought about an increased release of prostaglandins when administered to an isolated perfused rabbit kidney (78). The catecholamine-induced release of prostaglandins was thought to be mediated by an alpha receptor since phenoxybenzamine, but not propranolol, blocked this effect. Renal ischemia also stimulated the release of prostaglandins, an effect blocked by indomethacin but not phenoxybenzamine or propranolol (5). This latter effect supported the findings of McGiff et al. (65), who earlier observed increases in prostaglandin-like material from the canine kidney during ischemia. The increase in prostaglandin release observed in this study was evident not only in the ischemic but also the contralateral kidney. It was suggested that the increased production of angiotensin II from the ischemic kidney acted as a stimulus for prostaglandin synthesis in the contralateral kidney. Support for this latter proposal was given when angiotensin II injected into the renal artery increased the release of a prostaglandin-like substance from the dog kidney (66). While the previously mentioned stimuli all had the potential for producing relative renal ischemia, the final

common mechanism leading to activation of the biosynthesis of prostaglandins could not be attributed solely to this one action. Elevated renal vascular resistance was interpreted as the stimulus for prostaglandin release in Dunham and Zimmerman's report (20). The direct effect of catacholamines on prostaglandin synthesis was attributed to increased prostaglandin secretion in McGiff's studies (65).

In contrast to vasoconstrictor substances, an increased concentration of a PGE-like substance in renal venous blood occurred during the infusion into the renal artery of a vasodepressor agent (68). Bradykinin infusion increased the concentration of a PGE-like substance in renal venous blood from a mean control level of 0.16 ng/ml to 1.05 ng/ml. This increase occurred simultaneously with the greatest increase in renal blood flow to 423 ml/min from a control value of 282 ml/min. It was suggested from these results that PGE_2 participated in the renal vasodilator action of bradykinin. McGiff tested this proposal by using indomethacin to define the degree of dependency of the renal vasodilator action of bradykinin on its capacity to release PGE_2 from the kidney (67). The results demonstrated that the renal vasodilator action of bradykinin was in part due to the intrarenal release of PGE_2.

As stated previously, PGE_2: 1) is the most abundant renal prostaglandin and is a potent vasodilator; 2) affects

renal vascular resistance in subnanogram concentrations and is released in response to changes in perfusion pressure, renal blood flow and nerve stimulation, and 3) is directly stimulated by hormonal factors such as epinephrine, norepinephrine, bradykinin, and possibly angiotensin II. Due to these characteristics, PGE_2 has been considered to be a valid candidate for mediating renal autoregulation.

Interaction Between the Renin-angiotensin and Prostaglandin Systems

A working hypothesis for the hormonal regulation of renal circulation was proposed by Schmid in 1962 (86). He considered the renin-angiotensin system as an important factor involved in the regulation of intrarenal hemodynamics. Thurau proposed another theory supportive of Schmid's in which renal autoregulation was dependent upon the balance of a negative feedback system existing within the renin-angiotensin system (95). This negative feedback was in turn regulated by the concentration of sodium reaching one site of renin synthesis and release, known as the macula densa. While other reports supported the possibility of the existence of such a system (32,60), the data obtained from subsequent studies proved to be inconsistent with the hypothesis that the autoregulation of renal vascular resistance was the result of small changes in the endogenous intrarenal production and effect of angiotensin (9,23, 85).

Evidence of interaction between the renin-angiotensin and prostaglandin systems in regard to renal blood flow has lead to the suggestion by many investigators that the actions of both systems may interact in such a manner as to influence the regulation of renal circulation. It might, therefore, be expected that changes in the activity of one system would effect not only renal blood flow, but also the activity of the other system. Such an association was demonstrated in one study where the renin-angiotensin system was shown to activate prostaglandin synthesis during ischemia (94). In another report PGE_2 and angiotensin I contributed in a complimentary fashion to the regulation of fractional distribution of renal blood flow (45). Physiological antagonism between the two systems was noted when indomethacin produced a sharp reduction of resting renal blood flow (61), while inhibition of the activity of the renin-angiotensin system increased renal blood flow when renin release was experimentally elevated (26). Furthermore, the infusion of renin substrate into the isolated dog kidney (44) caused a redistribution of renal blood flow opposite to that seen upon the infusion of arachidonic acid (46). It was also shown that intrarenal generation of PGE_2 in response to angiotensin II attenuated the increase in renal vascular resistance caused by angiotensin II. These reports indicate that the renin-angiotensin system may directly

stimulate intrarenal prostaglandin secretion and in so doing antagonize its own action. The aforementioned studies also give support for the existence of a physiological type of feedback system acting to maintain the integrity of renal blood flow.

While evidence indicates that the renin-angiotensin system may directly stimulate prostaglandin secretion, further studies were conducted to determine the effect, direct or indirect, of intrarenal prostaglandins on the renin-angiotensin system. An in vitro study demonstrated that prostaglandins A_1 and A_2 act to competitively inhibit the renin reaction (55). Evidence from another report implied that prostaglandin-induced electrolyte and water loss stimulated the renin-angiotensin system (104). Furthermore, one report indicated that the infusion of prostaglandins into the renal artery of anesthetized dogs produced no detectable effect on renin release (97). While differences in experimental design, methods and procedures may explain the inconsistencies observed in the previously mentioned literature, no conclusions concerning the effect of prostaglandins on the renin-angiotensin system may be made at the present time.

Since the effects of the renin-angiotensin and prostaglandin systems were shown to be antagonistic insofar as renal blood flow was concerned, it was also of interest to

quantify these actions concerning sodium and water excretion. It is well-known that angiotensin II stimulates the secretion of aldosterone from the adrenal gland thereby indirectly causing an increased reabsorption of sodium and decreased excretion of water (19,76). Angiotensin II may also be expected to indirectly increase sodium reabsorption by decreasing blood flow to the cortex causing a decreased perfusion of the outer nephrons. In contrast, urine flow and sodium excretion may be increased when prostaglandin secretion is stimulated (40,66). A similar diuretic effect is obtained when PGE_1 is infused into the renal artery of anesthetized dogs (49). This apparent diuretic action of PGE_2 or PGE_1 may be attributed to the vasodepressor action of renal prostaglandins to increase blood flow throughout the cortex (61,14) or somewhat selectively in superficial areas (54) and therefore enhance the perfusion of outer nephrons. This action would then compromise the concentrating mechanism of the kidney. Other reports suggest a direct action of renal prostaglandins on specific sites within the nephron to decrease sodium reabsorption while increasing water loss (31,63). These studies have designated the proximal tubule and collecting tubules as possible sites of action of the hormone. Another mechanism for the diuretic action of prostaglandins was proposed when Anderson et al. (2), observed an antagonism between vasopressin and

prostaglandin occurring in vivo in the mammalian kidney. His results implicated a physiological role of prostaglandins in modulating the hydroosmotic effect of vasopressin in the mammalian kidney. The notion that PGE_2 acts as a significant intrarenal accelerator of sodium excretion was discouraged by Tobian et al. (96), who had shown that high sodium intake significantly reduced the intrarenal PGE_2 level rather than increasing it. This observation, however, may be due to the interaction of the renin-angiotensin system in which a high sodium load would decrease renin secretion, consequently removing the stimulation needed to increase prostaglandin synthesis.

Evidence presented thus far tends to support the hypothesis that intrarenal prostaglandins and the renin-angiotensin system interact in a complimentary manner to cause a physiological regulation of renal circulation. The existence of this interaction however, cannot be considered a certainty due to the limited conditions imposed upon these systems and evidence contradicting this proposal. Such contradictory evidence was presented when inhibitors and alterations imposed on the renin-angiotensin system had little effect on the autoregulatory response of the kidney (9,23,85).

Prostaglandins and Autoregulation

Several studies have demonstrated that reductions in renal blood flow following renal vasoconstriction is partially reversed coincident with elevated prostaglandin levels in renal venous blood (65,66). It was also shown that reactive hyperemia following release of renal arterial occlusion was decreased or abolished after indomethacin (40). As mentioned previously, inhibition of prostaglandin synthesis reduced renal blood flow and this reduction was closely correlated with a decline in the renal efflux of a substance having the properties of PGE_2 (61). From this study it was concluded that PGE_2 participates in maintaining renal vascular tone which before this was ascribed to autonomous, intrinsic renal arteriolar activity.

Because of these findings and others producing similar results, a role for prostaglandins in renal autoregulation was suggested. If autoregulation in response to reduced perfusion pressure could be attributed to redistribution of blood flow toward the inner regions of the kidney then it was feasible that this redistribution could be due to the action of intrarenal prostaglandins. Such a finding was reported in studies which observed distribution of renal blood flow in response to hemorrhage and vasopressor agents (70,83). Direct evidence concerning the possible role of prostaglandins in autoregulation was produced when

autoregulation of renal blood flow was abolished following the administration of indomethacin (39). Another study demonstrated that PGE_2 reversibly inhibited the noradrenaline overflow resulting from nerve stimulation of the rabbit kidney (24). From the results of this study it was proposed that endogenous prostaglandin controls norepinephrine release primarily from inner cortical nerve endings thereby maintaining juxtamedullary blood flow under periods of increased sympathetic nerve activity. While these reports support the role of prostaglandins in renal autoregulation, interpretation of results from other studies have produced opposite conclusions. One particular study observed that indomethacin had little or no effect on recovery of creatinine clearance after hemorrhage or on autoregulation of renal blood flow after alterations of renal perfusion pressure (10). Satah and Zimmerman reported that renal blood flow and renal vascular resistance were not significantly altered by infusion of meclofenamate into the renal artery of anesthetized dogs (84). It was also demonstrated that while indomethacin clearly decreased renal reactive hyperemia, its effect on the autoregulatory response suggested only minimal participation of prostaglandins in renal blood flow regulation (80). The inconsistent evidence presented thus far concerning the influence of prostaglandins on autoregulation does not allow us to assign to them

responsibility for the autonomous and intrinsic regulation of renal circulation.

Antihypertensive Function of Prostaglandins

The possibility that renal prostaglandins function as antihypertensive hormones was suggested in the findings of Strong et al. (90), when patients with renovascular hypertension were shown to have elevated levels of prostaglandin and renin. As antihypertensive agents, prostaglandins may exert their effect through one or more of three actions. PGE, possess vasodilator properties and the ability to suppress vascular responsiveness to endogenous constrictor amines and polypeptides (103). PGF compounds were observed to augment sympathetic nervous system activity and thereby increase the hypertensive state (13). Injection of PGE_2 inhibited the sympathetic neurotransmission in the perfused rabbit heart (37) and the release of norepinephrine from sympathetic nerves in the isolated perfused cat spleen (36). Finally, as previously mentioned, renal prostaglandins have shown the capability of producing a diuretic effect and thereby the ability to relieve a hypertensive condition brought about by hypervolemia.

Since some cases of hypertension have been due to excessive amounts of circulating angiotensin II, it was only reasonable to expect that another cause of hypertension may be due to low circulating levels of prostaglandins. It was

seen, however, that experimentally induced hypertension produced by renal ischemia initially increased both renin and prostaglandin levels during the acute phase of hypertension after which renin levels dropped while prostaglandin levels remained elevated (89). In contrast, Zusman et al. (109), demonstrated that in patients with essential hypertension or those with renal artery stenosis, PGA levels were statistically lower than levels observed in the control group. Transplants of fragmented renal medulla and a tissue culture of renomedullary interstitial cells were also shown to exert an antihypertensive effect on experimentally made hypertensive rats (74). It was previously mentioned that these cells and tissues contain large amounts of prostaglandins. Furthermore, a prostaglandin-A-secreting tumor was observed to cause a remission of a long-standing hypertension in a case described by Zusman et al. (108). The tumor was eventually removed after which hypertension was reestablished.

Mimran et al., recently observed the effects of prostaglandin and angiotensin II inhibitors on renal blood flow and blood pressure (73). In this study, indomethacin induced a decrease in renal blood flow and an increase in blood pressure. The latter effect did not occur, however, when rats were bilaterally nephrectomized. The angiotensin inhibitor blunted the blood pressure effect and prevented the renal haemodynamic changes induced by indomethacin.

The overall evidence suggest that renal prostaglandins may participate not only as factors in the autoregulation of renal circulation, but also as antihypertensive agents necessary for the maintenance of a normal tensive condition. The mechanism in which renal prostaglandins participate in these actions may be associated to their interaction with the renin-angiotensin system.

RATIONALE

The present study attempts to quantify how alterations in the renin-angiotensin system and changes in prostaglandin secretion affect each other and how these systems might be involved in the vasodilation produced by furosemide. The renin-angiotensin and prostaglandin systems are illustrated in Figure 1 along with those agents employed in this study to inhibit or stimulate the various components of the two systems. It is proposed that determining the influence of the renin-angiotensin system and renal prostaglandins on the furosemide induced increase in renal blood flow will provide pertinent information directed toward the realization of the mechanism involved in the regulation of renal circulation.

Figure 1. Points at which the renin-angiotensin and prostaglandin systems will be stimulated or inhibited during the experiments in this study. ₹ denotes stimulation while -//- denotes inhibition.

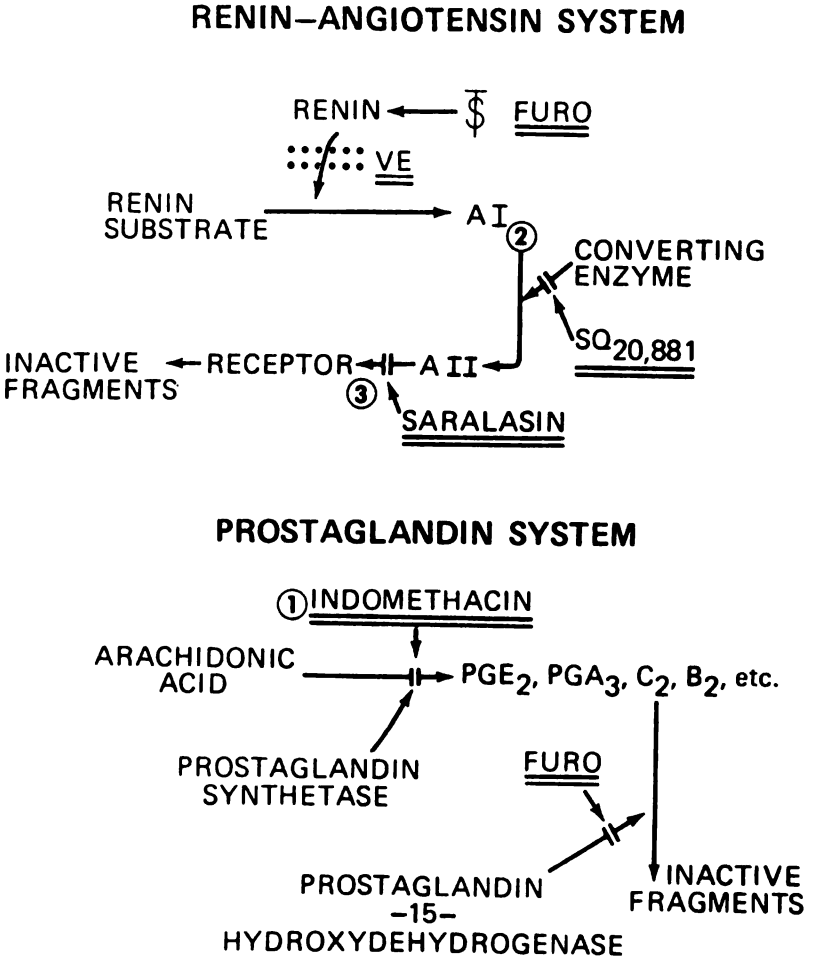

RENIN–ANGIOTENSIN SYSTEM
RENIN
FURO
VE
RENIN SUBSTRATE
AI
2
CONVERTING ENZYME
SQ20,881
INACTIVE FRAGMENTS
RECEPTOR
A II
3
SARALASIN
PROSTAGLANDIN SYSTEM
1 INDOMETHACIN
ARACHIDONIC ACID
PGE2, PGA3, C2, B2, etc.
PROSTAGLANDIN SYNTHETASE
FURO
PROSTAGLANDIN –15– HYDROXYDEHYDROGENASE
INACTIVE FRAGMENTS

Figure 1

METHODS

Animal Preparation

Male mongrel dogs were anesthetized with sodium pentobarbitol (30 mg/kg) and artificially ventilated (Harvard Apparatus Respirator). A polyethelene catheter was inserted through the left femoral artery into the abdominal aorta to collect arterial blood samples and monitor arterial blood pressure using a strain-gauge pressure transducer (Statham P 23 AA) and direct writing oscillograph (Grass Polygraph). Polyethelene catheters were inserted into the left and right femoral veins for infusion of inulin and saline, respectively. After exposing the left kidney via a retroperitoneal flank incision both ureters were cannulated using polyethlene tubing and the left spermatic vein was tied. A curved 20-gauge needle attached to polyethelene tubing was inserted into the renal vein for collection of renal venous blood samples. A curved 22-gauge needle attached to polyethelene tubing was inserted into the renal artery for the infusion of saline or drugs. Total renal blood flow from one kidney was recorded using a non-cannulating electromagnetic flowmeter (Carolina Instruments Electromagnetic) which was placed on the renal artery.

A hilar lymph vessel was isolated and cannulated with polyethelene tubing (PE-50).

Approximately one hour prior to the first collection period an intravenous infusion of a 3% inulin solution was initiated at a rate of 1 ml/min and a 0.9% saline solution was infused into the renal artery at a rate of 1.1 ml/min. Maintenance doses of anesthetic were administered subcutaneously throughout the experiment as needed. At the end of each experiment the left kidney was removed and weighed.

Experimental Protocol

Duplicate samples of urine, lymph, and arterial and renal venous blood were taken during each period of the experiment. Urine and lymph samples were collected over twenty minute periods. Arterial and renal venous blood samples were drawn at the midpoint of the collection of urine and lymph. Half of the blood drawn from the renal vein was transferred to chilled test tubes containing disodium ethylenediaminetetraacetic acid (EDTA) while the other half was transferred to test tubes and allowed to clot at room temperature for twenty minutes. The clot was then dislodged from the sides of the test tube and the sample centrifuged for ten minutes, after which the serum was drawn off and collected in chilled test tubes. Lymph was collected in chilled tubes containing EDTA.

After the animals were allowed a minimum of one hour to recover from surgery, the following protocols were undertaken.

Protocol I: In six dogs, urine, lymph, and arterial and renal venous blood samples were collected during a control period. The dogs were then volume expanded with isotonic saline infused into the femoral vein at a rate of 1 ml/kg/min for thirty minutes and maintained at an infusion rate of 5 ml/min above the minute urine flow rate for one hour and samples of urine, plasma and lymph were again collected. Furosemide (0.015 mg/kg/min) was then infused into the renal artery. Five to ten minutes were allowed for adjustment of the saline infusion to a rate of 5 ml/mkn above the increase minute urine flow. After renal blood flow and urine minute volume stabilized samples were again collected.

Protocol II: In seven dogs, urine, lymph, and arterial and renal venous blood samples were collected during the first two periods under conditions (control and volume expanded) described above. A 3.5 mg/kg bolus of indomethacin was then injected into the femoral vein over a five minute period. Another five minutes was allowed for the readjustment of the saline infusion to 5 ml/min above the urine flow rate. After renal blood flow and blood pressure had stabilized, samples were collected as before.

Furosemide was then infused into the renal artery at the previously described concentration and rate and the saline infusion rate again adjusted. Samples were collected following the stabilization of renal blood flow and minute urine volume.

Protocol III: In five dogs, urine, lymph, and arterial and renal venous blood samples were collected during the first two periods under conditions in Protocols I and II. SQ 20,881 (1 mg/kg) was then administered into the femoral vein over a five minute period. Another five minutes were allowed for the readjustment of the saline infusion to five ml/min above the urine flow rate. After renal blood flow and blood pressure had stabilized, samples were collected as before. A final collection period following the infusion of furosemide was performed under the same conditions described in the previous two protocols.

Protocol IV: In five dogs, urine, lymph, and arterial and renal venous blood samples were collected during the first two periods under conditions previously described. Saralasin was then infused into the renal artery at a rate of 0.5 μg/kg/min for thirty minutes. The efficacy of saralasin infusion was evaluated by first injecting angiotensin II (0.005 mg) into the renal artery in order to cause a significant decrease in renal blood flow. This procedure was repeated five minutes after the initiation of the

saralasin infusion and in all cases the effect of angiotensin II on renal blood flow was completely inhibited. Following the saralasin infusion a solution of furosemide and saralasin was infused into the renal artery at the concentrations and rates previously mentioned.

Protocol V: In seven dogs, urine, lymph, and arterial and renal venous blood samples were collected during a control period. Furosemide was then infused into the renal artery at its previous concentration and rate. Samples were collected following the stabilization of all parameters mentioned previously.

Analytical Methods

Arterial and renal venous plasma and urine samples were analyzed for inulin concentration using the diphenylamine method of Walser, Davidson, and Orloff (102). Renin activity in femoral arterial and renal venous blood samples was determined by the method of Haber et al. (33), using a renin activity radioimmunoassay for angiotensin I. Renin secretions were calculated as the product of the renal venous-arterial renin activity difference and renal plasma flow, and expressed as nanograms secreted per minute (ng/min). Renal lymph and venous prostaglandin E_2 concentration was measured in renal venous serum and lymph using a radioimmunoassay for PGE_2 developed by Stygles et al. (91). Prostaglandin samples of the control experiments,

experiment I, in which furosemide was infused following volume expansion were first extracted with chloroform and later assayed with the use of an antibody provided by the Upjohn Co. The cross reactivity of this antibody with other prostaglandins was high and specificity low. Another antibody specific for PGE_2 was then developed in this laboratory using thyroglobulin as the hapten. This antibody was highly specific for PGE_2 while showing low cross reactivity with herterologous prostaglandins. The antibody was then used in the aforementioned radioimmunoassay of unextracted serum samples collected from the remaining experiments.

Tubes for measuring the concentration of PGE_2 in each sample were prepared containing 100 µl of sample, 100 µl of ^3H-PGE_2 containing 10,000-12,000 cpm/ml and 0.5 ml of an appropriate dilution of antibody. The dilution of antibody was considered appropriate when it bound 41-50% of ^3H-PGE_2. The tubes were then mixed and allowed to equilibrate at 4°C for at least 18 hours after which ^3H-PGE_2 bound to antibody was separated from unbound ^3H-PGE_2 by the addition of 0.5 ml of a dextran charcoal solution (0.25 mg/ml Dextran T-70: 2.5 mg/ml Norit A Charcoal). Tubes were then spun at 3000 rpm at ambient temperature for 20 minutes. The supernatant was decanted into glass scintillation vials, 7.5 ml aquasol (New England Nuclear) added, the vials mixed and counted to 2% error in a Beckman LS-100 liquid scintillation counter.

The assay was shown to be useful between 0.01 and 10.0 ng/ml PGE_2.

While arterial PGE_2 concentration was shown to be measurable by our assay, the low values obtained displayed a minimal amount of variation throughout the experiment and were therefore treated as a constant, unnecessary in the calculation of renal venous secretion.

Secretion rates for PGE_2 were calculated as the product of serum concentration and renal plasma flow and expressed as nanograms secreted per minute (ng/min). Blood pressure (BP) and renal blood flow (RBF) were obtained directly from the recordings, and renal plasma flow (RPF) calculated from the blood flow and hematocrit (RPF=RBF x 1-Hct.). Renal resistance (RR) was calculated from the mean systemic blood pressure and total renal blood flow: RR (mmHg/ml/min) = BP/RBF. Hematocrit was determined on all arterial blood samples by the micro method. Glomerular filtration rate was estimated from the clearance of inulin. Sodium concentration in urine was determined by flame photometry (Instrumentation Laboratories). Sodium excretion was calculated as the product of urine sodium concentration and minute urine volume and expressed as micro equivalents per minute (μeq/min).

Renin and PGE_2 secretion had significant heterogenity of variance by Bartlett's test for homogeneity (88).

Because of this, data was plotted on semi-logarithmic graphs and statistical analysis performed on the logarithmic transformation. All data was analyzed using either the randomized complete block or completely randomized design analysis of variance (88). The least significant difference test was used in the comparison of means (88). The 0.05 level of probability was used as the criterion of significance.

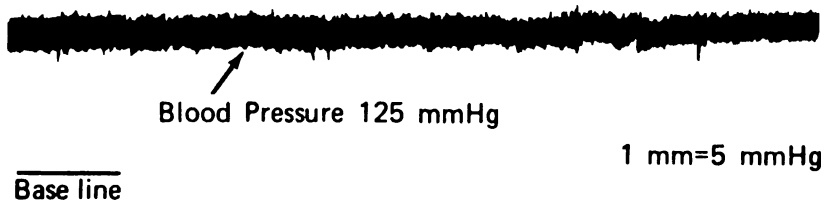
RESULTS

The typical effect of furosemide on renal blood flow in volume expanded dogs is illustrated in Figure 2. The response is characterized by a transient decrease followed by a sustained increase in renal blood flow. The latter effect is maintained as long as the infusion is continued. Blood pressure is shown to be unaffected by this response. For the purpose of comparison of results, animals receiving furosemide after volume expansion will be considered as controls. Animals receiving treatment following the volume expansion period and preceding the infusion of furosemide will be identified according to the treatment received.

Figure 3 illustrates the effect of indomethacin on the increase in renal blood flow induced by furosemide. Renal blood flow shown in this and all similar figures will henceforth be expressed as changes from the control period. Renal blood flow was unaltered by volume expansion in the control or indomethacin treated group. In six control animals, furosemide induced a mean increase in renal blood flow of 144 $\pm$ 28 ml/min. In nine animals indomethacin had no effect on renal blood flow and reduced the furosemide response in which an increase of only 52 $\pm$ 28 ml/min was

Figure 2. Typical effect of furosemide on renal blood flow and blood pressure in volume expanded dogs.

FUROSEMIDE CONTROL



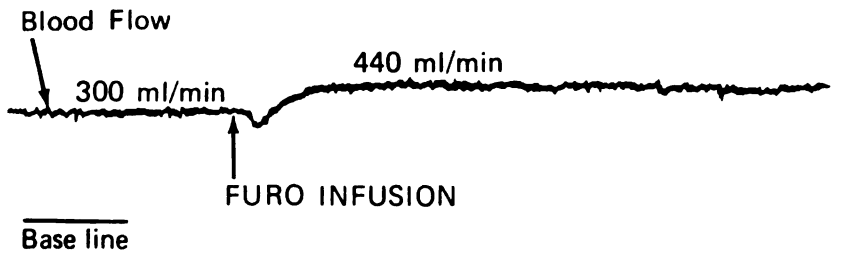


Figure 2

Figure 3. Effects of volume expansion (VE), treatment (Trt) and furosemide (F) on renal blood flow in the furosemide control and indomethacin treated groups. Values are expressed as changes from the control period. Mean $\pm$ sem are shown. A significant increase ($P < 0.05$) in renal blood flow was noted in both groups following furosemide. A significant difference was noted between the furosemide control and indomethacin treatyd groups during the furosemide period.

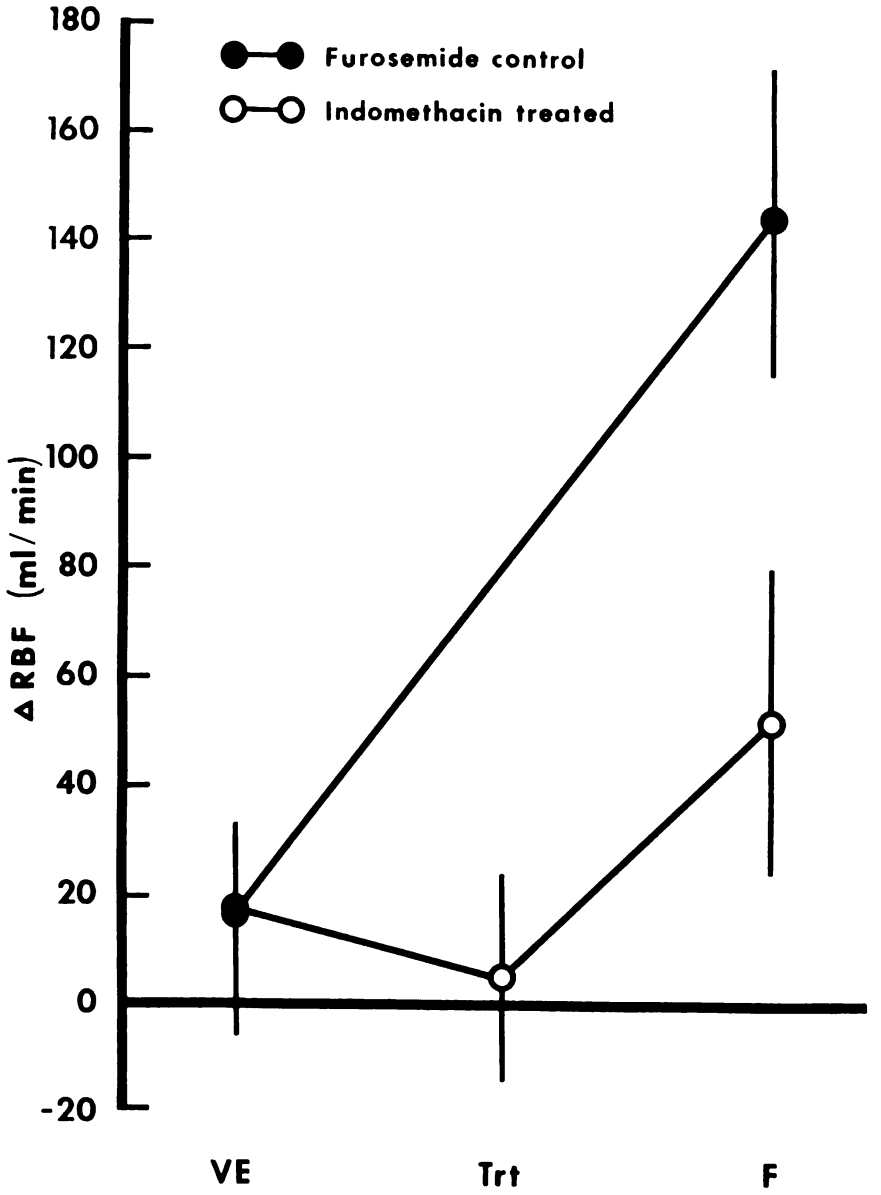

180
160
140
120
100
80
60
40
20
0
-20
Furosemide control
Indomethacin treated
Δ RBF (ml/min)
VE
Trt
F

Figure 3

observed. While both groups demonstrated an increase in renal blood flow following furosemide, the increase observed in the indomethacin group was significantly less than that of the control.

PGE_2 secretion rates in each experimental period of the control and indomethacin treated groups are shown in Figure 4. PGE_2 secretion was unaltered by volume expansion in either group. In the control group, furosemide induced a significant mean increase in PGE_2 secretion of 440 $\pm$ 328 ng/min above the volume expansion period. Indomethacin diminished PGE_2 secretion to low levels which were maintained following furosemide.

Figure 5 illustrates the effect of SQ 20,881 on the increase in renal blood flow induced by furosemide. In five animals renal blood flow was unaltered by either volume expansion or SQ 20,881 injection. The increase in renal blood flow induced by furosemide in the control group was blunted in the SQ 20,881 group showing an increase of 76 $\pm$ 29 ml/min.

Figure 6 shows the PGE_2 secretion rates in each experimental period of the control and SQ 20,881 treated groups. Neither volume expansion nor SQ 20,881 injection was shown to significantly alter PGE_2 secretion. The furosemide induced increase in PGE_2 secretion observed in the control group was not demonstrated in animals pretreated with SQ 20,881.

Figure 4. PGE_2 secretion rates in the control (C), volume expansion (VE), treatment (Trt) and furosemide (F) periods of the furosemide control and indomethacin treated groups. Mean $\pm$ sem are shown. A significant increase ($p < 0.05$) in PGE_2 secretion was observed following furosemide in the control group. A significant decrease in PGE_2 secretion was observed following treatment with indomethacin.

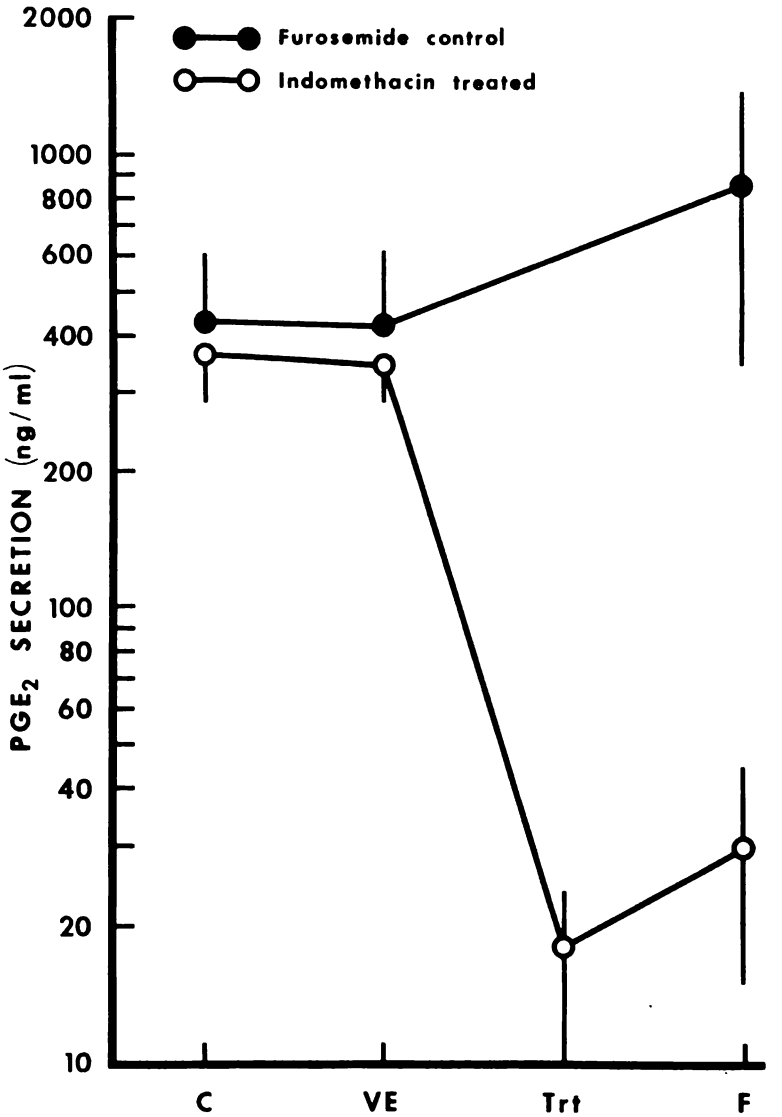

Furosemide control
Indomethacin treated
2000
1000
800
600
400
200
100
80
60
40
20
10
PGE2 SECRETION (ng/ml)
C
VE
Trt
F

Figure 4

Figure 5. Effects of volume expansion (VE), treatment (Trt) and furosemide (F) on renal blood flow in the furosemide control and SQ 20,881 treated groups. Values are expressed as changes from the control period. Mean ± sem are shown. A significant increase ($p < 0.05$) in renal blood flow was noted in both groups following furosemide.

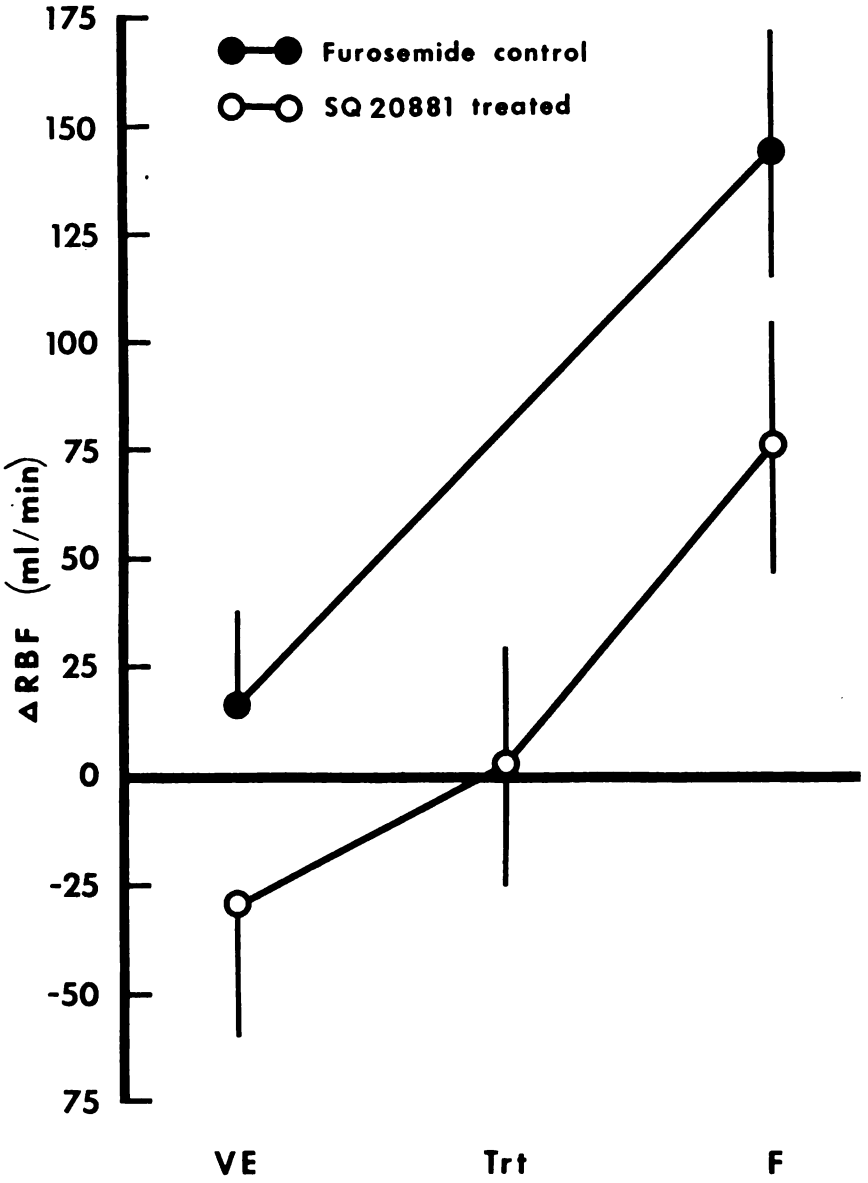

175
150
125
100
75
50
25
0
-25
-50
75
ΔRBF (ml/min)
Furosemide control
SQ 20881 treated
VE
Trt
F

Figure 5

Figure 6. PGE_2 secretion rates in the control (C), volume expansion (VE), treatment (Trt) and furosemide (F) periods of the furosemide control and SQ 20,881 treated groups. Mean + sem are shown. A significant increase ($p < 0.05$) in PGE_2 secretion was observed following furosemide in the furosemide control group.

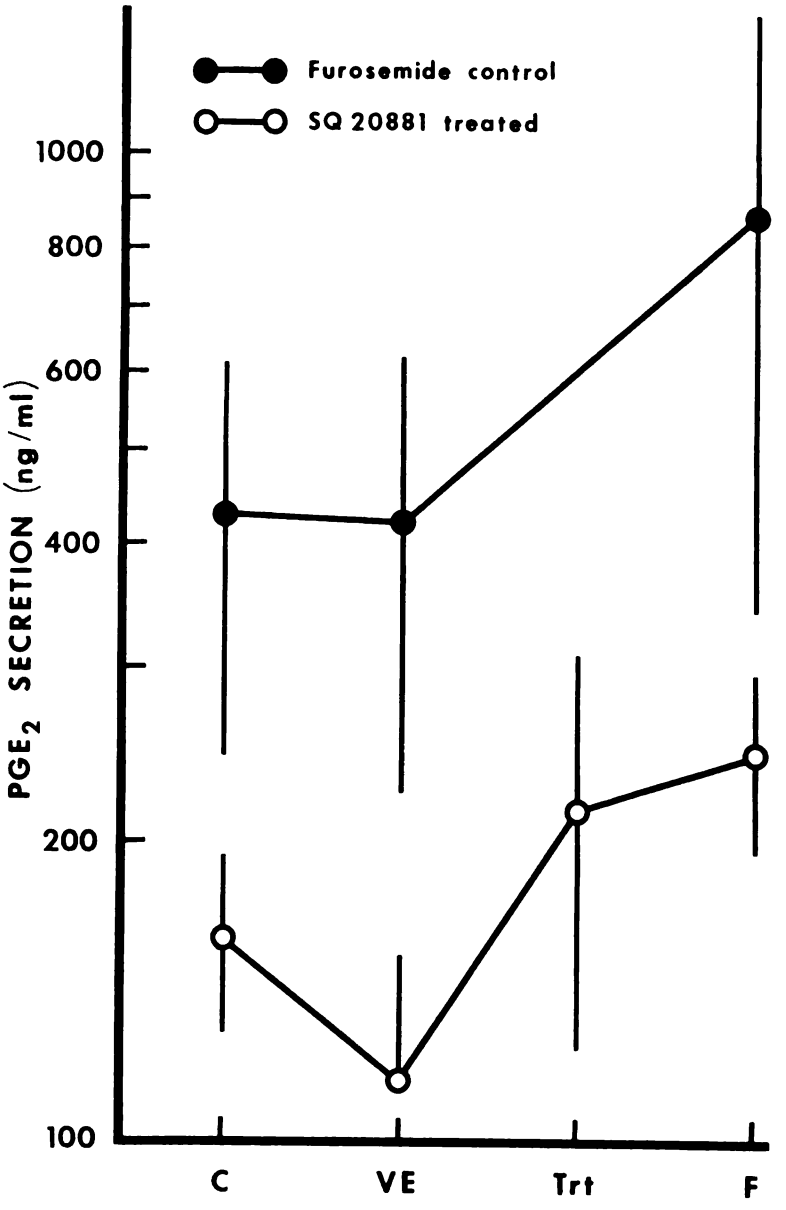

Furosemide control
SQ 20881 treated
1000
800
600
400
200
100
PGE2 SECRETION (ng/ml)
C
VE
Trt
F

Figure 6

In each experimental period, significant differences in renal blood flow were not observed when four animals treated with 1-Sar-8-Ala-Angiotensin II, saralasin, were compared to control animals (Figure 7). Following furosemide an increase in renal blood flow of 193 $\pm$ 55.3 ml/min was observed in animals treated with saralasin. This increase was not significantly different than that seen in the control group. A similar trend was noted when PGE_2 secretion rates of the saralasin treated and control animals were determined (Figure 8). While volume expansion slightly depressed PGE_2 secretion in the saralasin group, furosemide caused a mean increase in secretion over the volume expanded period of 325 $\pm$ 95 ng/min. The aforementioned increase induced by furosemide was not found to be significantly different than that of the control group.

As previously mentioned, the increase in renal blood flow caused by furosemide was significantly reduced by pretreatment with indomethacin (Figure 3). It was observed, however, that indomethacin did not abolish the furosemide response. It is the latter effect which has directed further studies toward determining the influence of other factors on the furosemide response. Due to its action to increase the diuretic effect of furosemide and decrease renin secretion, volume expansion was evaluated as one such factor.

Figure 7. Effects of volume expansion (VE) and furosemide (F) on renal blood flow in the furosemide control and saralasin treated groups. Values are expressed as changes from the control period. Mean ± sem are shown. A significant increase ($p < 0.05$) in renal blood flow was noted in both groups following furosemide.

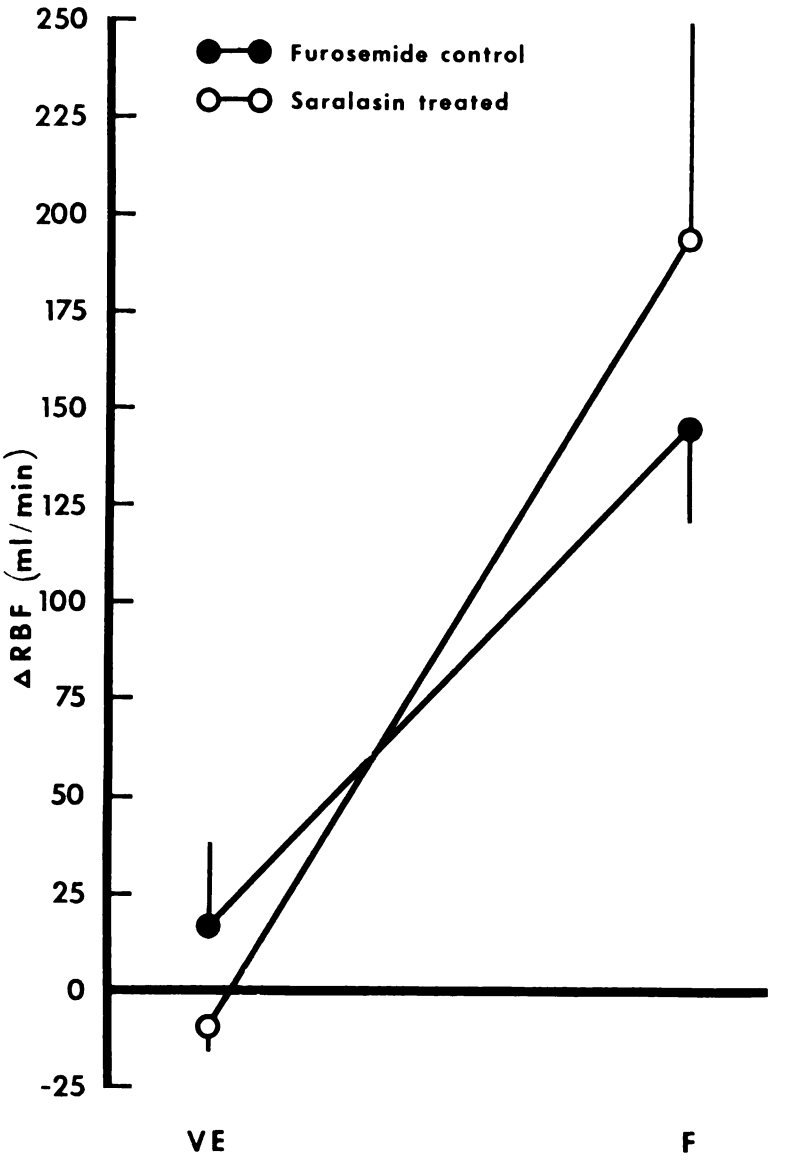

250
225
200
175
150
125
100
75
50
25
0
-25
ΔRBF (ml/min)
Furosemide control
Saralasin treated
VE
F

Figure 7

Figure 8. PGE_2 secretion rates in the control (C), volume expansion (VE) and furosemide (F) periods of the furosemide control and saralasin treated groups. Mean $\pm$ sem are shown. A significant increase ($p < 0.05$) in PGE_2 secretion was observed following furosemide in both groups.

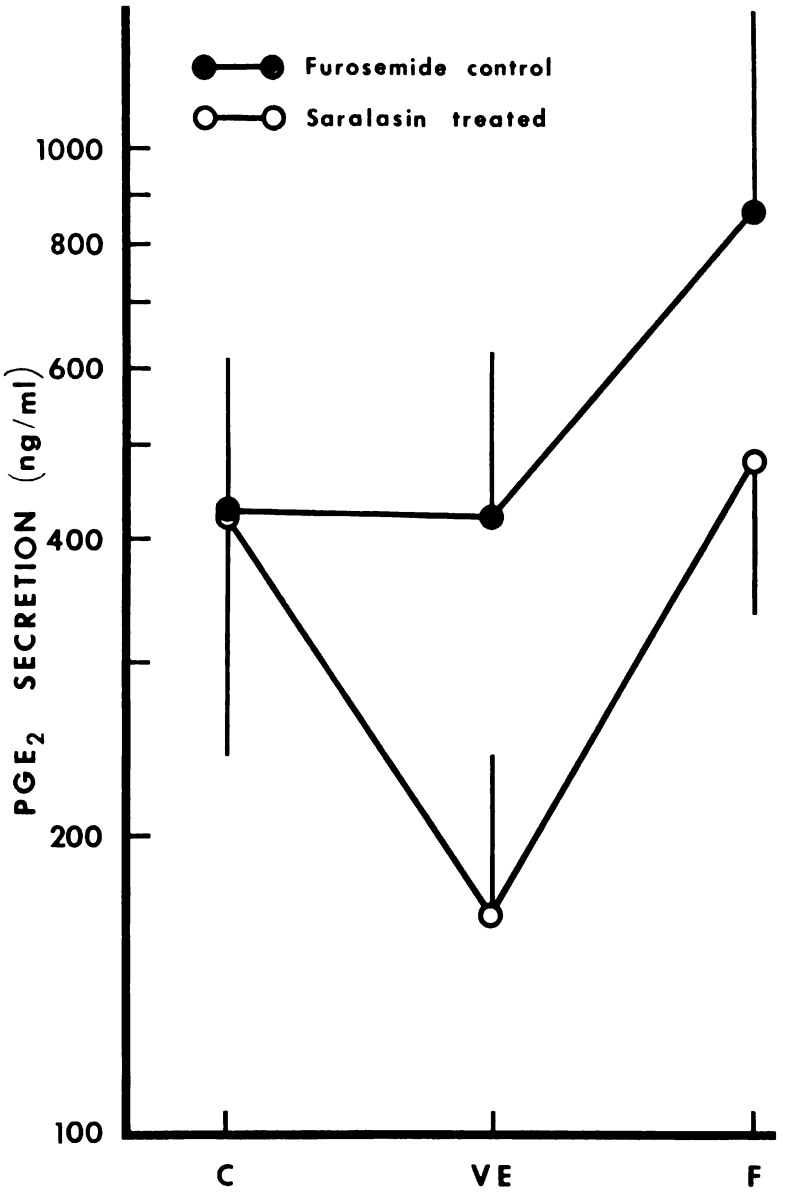

Furosemide control
Saralasin treated
1000
800
600
400
200
100
PGE2 SECRETION (ng/ml)
C
VE
F

Figure 8

Figure 9 illustrates the comparison of mean values of renal blood flow between animals not subjected to volume expansion, hydropenic, and control animals following furosemide. Animals were considered hydropenic if their hematocrit was above 40% and their total minute urine volume was below 0.5 ml/min. In seven hydropenic animals, no effect on renal blood flow was observed following furosemide. A similar trend was noted when PGE_2 secretion rates were determined in hydropenic animals (Figure 10). Furosemide produced no effect on PGE_2 secretion in the hydropenic animals.

To further investigate the mechanism through which furosemide acts to increase renal blood flow, a small amount of data on hilar lymph renin secretion and PGE_2 concentration was obtained (Table I). One or more experiments from the control, indomethacin, and SQ 20,881 groups revealed that volume expansion decreased and furosemide infusion increased hilar lymph renin secretion. In one control experiment, lymph PGE_2 concentration was decreased during volume expansion and increased following furosemide. In two indomethacin and one SQ 20,881 experiments, PGE_2 concentration decreased during volume expansion and remained unchanged over the treatment and furosemide periods.

Absolute values of the various parameters measured in one control and one indomethacin treated experiment are

Figure 9. Effects of furosemide on renal blood flow in the furosemide control and hydropenic groups. Values are expressed as changes from the control period. Mean ± sem are shown. A significant increase ($\bar{p} < 0.05$) in renal blood flow was noted in the furosemide control group.

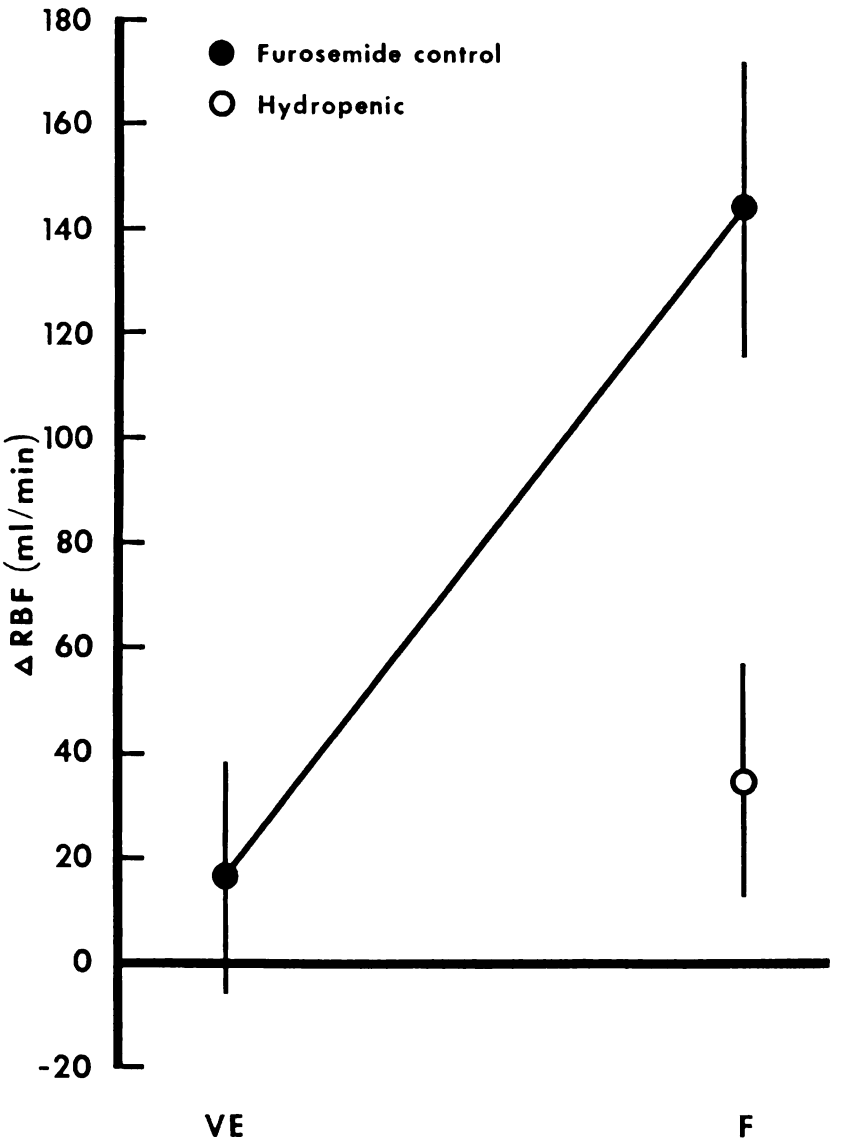

180
160
140
120
100
80
60
40
20
0
-20
Furosemide control
Hydropenic
ΔRBF (ml/min)
VE
F

Figure 9

Figure 10. PGE_2 secretion rates in the control (C), volume expansion (VE) and furosemide (F) periods of the furosemide control and hydropenic groups. Mean $\pm$ sem are shown. A significant increase ($p < 0.05$) in PGE_2 secretion was observed following furosemide in the furosemide control group.

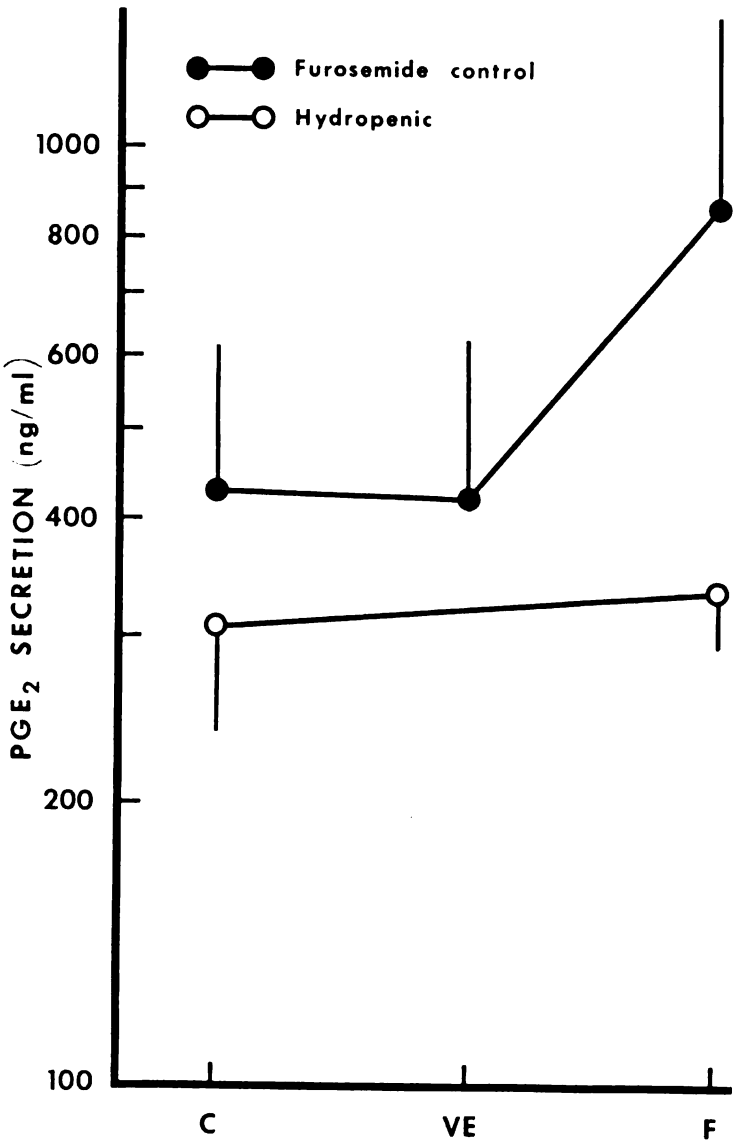

Furosemide control
Hydropenic
1000
800
600
400
200
100
PGE2 SECRETION (ng/ml)
C
VE
F

Figure 10

Table I. Hilar lymph renin secretion and prostaglandin concentration in each experimental period of the furosemide control, indomethacin treated and SQ 20,881 treated groups.

Experiment	n	Control	Volume Expansion	Treatment	Furosemide
		Lymph Renin Secretion ng/min			
Furosemide Control	4	5.6 ± 0.14	1.9 ± 0.8		12.8 ± 2.2
Indomethacin Treated	3	1.54 ± 0.14	0.57 ± 0.01	0.64 ± 0.21	1.82 ± 0.22
SQ 20,881 Treated	1	1.9	0.24	0.09	47
		Lymph PGE_2 Concentration ng/ml			
Furosemide Control	1	0.22	0.14		0.32
Indomethacin Treated	2	0.28 ± 0.01	0.05 ± 0.008	0.03 ± 0.005	0.05 ± 0.009
SQ 20,881 Treated	1	0.62	0.14	0.10	0.14

illustrated in Table II, and are representative of the mean values determined in each group. Volume expansion was shown to decrease renin secretion, and increase sodium excretion and minute urine volume in all experimental groups. It was also observed that prostaglandin synthesis and renin-angiotensin inhibitors did not significantly alter the increase in sodium excretion (Figure 11), renin secretion (Table II), or minute urine volume induced by furosemide. Significant changes in renal resistance paralleled corresponding changes in renal blood flow in all experimental groups. A summary of the results obtained from this study is expressed in Table III.

Table II. Absolute values of parameters measured in one furosemide control and one indomethacin treated experiment.

	R.B.F. ml/min	B.P. mmHg	R.R. p.r. units	Hct.	Na Ex. μeq/min	V ml/min	G.F.R. ml/min	Renin ng/min	PGE_2 ng/min
FUROSEMIDE CONTROL									
Control Period									
10:30	258	125	.48	40.0	3.7	.15	54.1	1014	186
10.50	262	125	.48	41.0	3.6	.16	49.4	464	433
Volume Expansion Period									
12:30	258	115	.45	34.0	447	2.1	61.4	0	365
12:50	267	110	.41	34.0	374	2.1	63.2	0	520
Furosemide Period									
1:25	394	110	.28	32.0	3020	15.6	54.6	1573	697
1:45	394	105	.27	32.0	3250	16.2	54.7	710	884
INDOMETHACIN TREATED									
Control Period									
10:30	281	105	.37	43.0	3.6	.08	55.1	6778	272
10:50	281	105	.37	43.0	4.2	.08	39.0	4101	401
Volume Expansion Period									
12:30	306	115	.38	33.5	126	.96	69.7	112	326
12:50	315	110	.35	33.5	130	.95	68.5	0	482
Treatment Period									
1:30	298	110	.37	33.0	125	1.05	64.3	0	0
1:50	298	105	.35	32.0	112	1.05	63.3	615	0
Furosemide Period									
2:25	323	105	.33	32.0	1074	9.3	54.6	4213	0
2:45	306	105	.34	32.0	842	11.3	63.1	1890	0

R.B.F. = renal blood flow
B.P. = mean systemic blood pressure
R.R. = renal resistance
Hct. = hematocrit
Na Ex. = sodium excretion
V = minute urine volume
G.F.R. = glomerular filtration rate
Renin = renin secretion
PGE_2 = prostaglandin secretion

Figure 11. Effects of volume expansion (VE), treatment (Trt) and furosemide (F) on sodium excretion in the SQ 20,881 treated, indomethacin treated and furosemide control groups. Values are expressed as changes from the control period. Mean $\pm$ sem shown. A significant increase ($p < 0.05$) in sodium excretion was noted following volume expansion, treatment and furosemide in all groups.

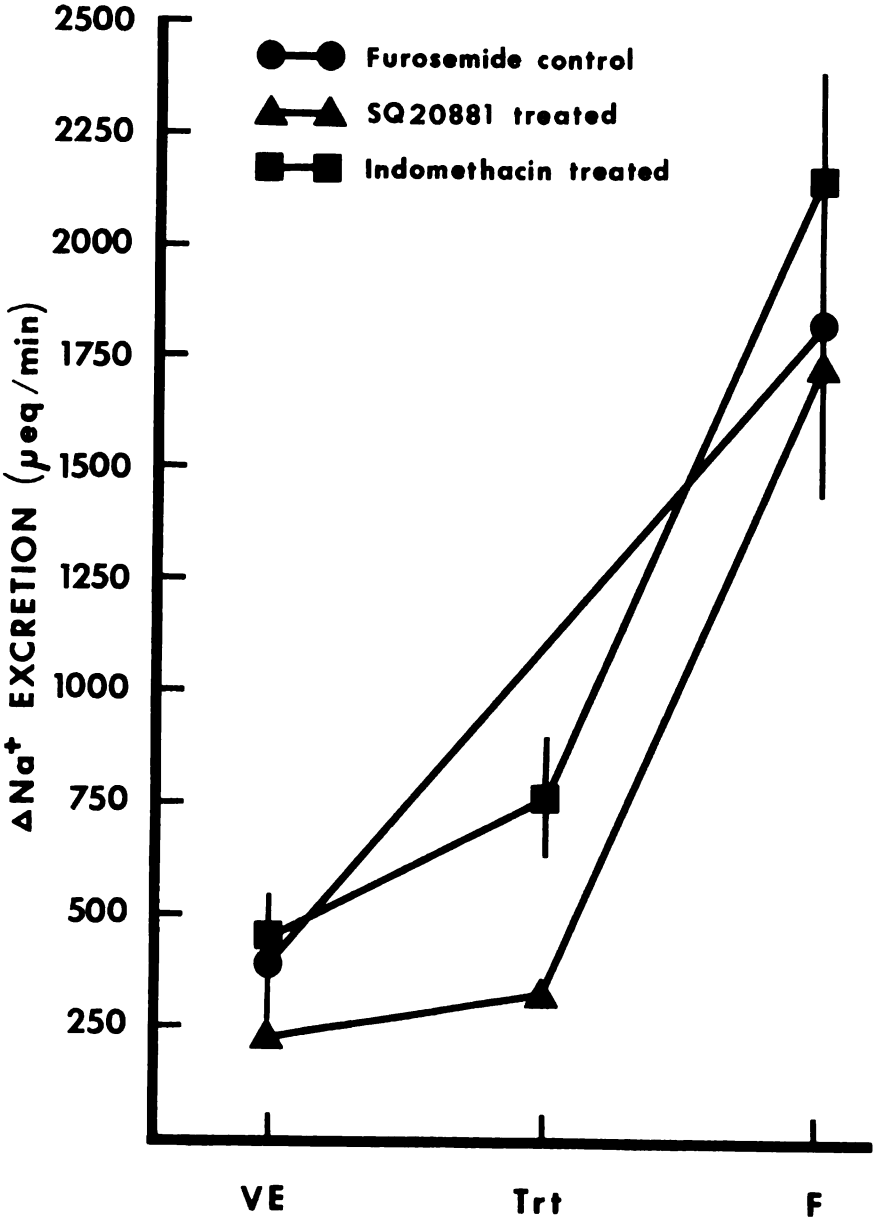

2500
2250
2000
1750
1500
1250
1000
750
500
250
ΔNa+ EXCRETION (μeq/min)
Furosemide control
SQ20881 treated
Indomethacin treated
VE
Trt
F

Figure 11

Table III. Summary of results presented in this study.

I.	Furosemide produces an increase in renal blood flow which is significantly reduced by pretreatment with indomethacin.
II.	Furosemide causes an increase in PGE_2 secretion which is inhibited by indomethacin.
III.	SQ 20,881 is shown to blunt the increase in renal blood flow induced by furosemide.
IV.	Increases in PGE_2 secretion following furosemide were not observed in animals pretreated with SQ 20,881.
V.	The increase in renal blood flow and PGE_2 secretion induced by furosemide was not significantly altered in animals treated with saralasin.
VI.	The effect of furosemide on renal blood flow and PGE_2 secretion was significantly greater in the volume expanded than in the hydropenic animals.
VII.	Volume expansion decreased renin secretion while PGE_2 secretion remained unaltered.
VIII.	Increases in renin secretion induced by furosemide were unaltered by prostaglandin synthesis or renin-angiotensin inhibitors.

DISCUSSION

Since the discovery of two intrarenal hormonal systems with potent vasoactive effects, the renin-angiotensin and prostaglandin systems, investigators have suggested that regulation of renal circulation may be subjected to hormonal influence. Tarrenbaum et al., reported that infusion of PGE_2 or arachidonic acid into the renal artery of anesthetized dogs produces an increased total renal blood flow (93). Furthermore, indomethacin inhibited the effects of arachidonic infusion while none of the effects produced by PGE_2 infusion were altered. Systemic injection of indomethacin was also shown to reduce and alter the distribution of renal blood flow in anesthetized dogs (61). Similar results were reported in a study by Itskovitz et al. (45), when they observed diminished inner cortical blood flow following indomethacin injection.

Other evidence has been obtained indicating angiotensin II as an intrarenal hormone acting to constrict the inner cortical and medullary vasculature (42). In support of this evidence, Itskovitz et al. (44), observed that an experimentally induced deficiency of renin substrate, and consequently angiotensin II, was associated with an increased

fraction of inner cortical blood flow. Infusions of renin substrate reestablished the fractional distribution of renal blood flow to that observed prior to induction of the deficiency. These data provide evidence to support the hypothesis that angiotensin might act as an intrarenal hormone which participates in the regulation of deep cortical and medullary vasculature resistance.

While both the renin-angiotensin system and prostaglandin system influence renal hemodynamics, the possible interaction between the two systems and effect of this interaction on renal blood flow may prove to be an important factor in the regulation of renal circulation. Aiken and Vane presented evidence supporting an interaction between the two hormonal systems when they observed an increased release of a PGE-like material from the kidney upon the infusion of angiotensin II into the renal artery (1). It was also shown that angiotensin II infusion into an isolated perfused kidney consistently decreased blood flow to the cortex, while its effects on blood flow to the inner cortex were variable (45). This latter action was thought to be related to the release of renal prostaglandins by angiotensin II, especially in those experiments in which angiotensin II increased inner cortical blood flow. Furthermore, an explanation for the tachyphylaxis to the renal vasoconstrictor actions of angiotensin II observed by

Louis and Doyle (62), was suggested from the interpretation of results presented in a study by McGiff et al. (64). Their results indicated that tachyphylaxis to angiotensin II is associated with the release of PGE_2 which opposes the vasoconstrictor-antidiuretic actions of this hormone.

The present study attempts to dissect further the intrarenal role of the renin-angiotensin and prostaglandin systems by evaluating the effects of renin-angiotensin and prostaglandin synthetase inhibitors on the increase in renal blood flow, prostaglandin secretion and renin secretion induced by furosemide. Furosemide was shown to induce a large increase in renal blood flow due to a decrease in renal resistance (Figure 3), while at the same time increase PGE_2 secretion from the canine kidney (Figure 4). These data suggest that the increase in renal blood flow and its corresponding decrease in renal resistance, is related to the increase in PGE_2 secretion. The effect of indomethacin on the increase in renal blood flow induced by furosemide gives further support to the existence of such a relationship (Figure 3). These results are consistent with those studies which found that furosemide did not alter renal blood flow in animals pretreated with indomethacin (8,106).

In addition to its natriuretic effect, furosemide has been shown to increase renin secretion (98), and in one report the diuretic was observed to inhibit prostaglandin-15-hydroxy dehydrogenase activity <u>in vitro</u> (81). Because of

these actions, it was proposed that furosemide may induce an increase in PGE_2 secretion in one of three ways: 1) by inhibition of prostaglandin-15-hydroxy dehydrogenase and subsequently increasing the half-life of intrarenal prostaglandins, 2) by a direct action of the diuretic itself, and/or 3) by stimulation of synthesis of prostaglandins via the renin-angiotensin system. Involvement of the renin-angiotensin system in the mechanism through which furosemide acts to increase PGE_2 secretion and renal blood flow was evaluated by observing the effects of volume expansion, SQ 20,881 injection, and saralasin infusion on PGE_2 secretion and renal blood flow both prior to and after the infusion of furosemide. Volume expansion decreased renin secretion in all experimental groups (Table II), while no effect on PGE_2 secretion or renal blood flow was observed (Figures 3-8). The apparent dissociation of activity between the two hormonal systems under conditions of volume expansion may be due to a tonic release of prostaglandins from the resting kidney, independent of the influence of low concentrations of substituents composing the renin-angiotensin system. This interpretation has considerable support since indomethacin has been observed to decrease resting renal blood flow (61), and diminish inner cortical blood flow in anesthetized dogs.

Systemic injection of the angiotensin converting enzyme inhibitor, SQ 20,881, in volume expanded dogs has no effect on either PGE_2 secretion or renal blood flow (Figures 5 and 6). The absence of an effect of SQ 20,881 on renal blood flow and PGE_2 secretion was expected since the activity of the renin-angiotensin system was already diminished due to the action of volume expansion on renin secretion. In animals pretreated with SQ 20,881, PGE_2 secretion was not significantly increased above the volume expansion period following furosemide (Figure 6). Furosemide did, however, significantly increase renal blood flow in the SQ 20,881 group, even though this increase was observed to be blunted when compared to that of the control group (Figure 5). The observation that an increase in renal blood flow is not accompanied by an increase in PGE_2 secretion is inconsistent with the previously mentioned proposal relating the increase in renal blood flow induced by furosemide to the corresponding increase in PGE_2 secretion. This apparent inconsistency, however, may be due to the inability of our assay to detect small changes in renal venous PGE_2 concentration.

In one study, Needleman et al. (77) reported that angiotensin I was only one-tenth to one-thirtieth as efficient as angiotensin II in releasing prostaglandins from the isolated perfused rabbit kidney. Results from another study showed that angiotensin I consistently decreased

inner cortical blood flow whereas angiotensin II consistently decreased blood flow to the outer cortex while its effects on blood flow to the inner cortex were variable (45). Interpretation of these results lead to the suggestion that the constancy of the vasoconstrictor action of angiotensin I on inner cortical blood vessels as compared with that of angiotensin II was related to the lesser ability of angiotensin I to release renal prostaglandins. This interpretation may also apply to the observations in the present study in which the release of PGE_2 following furosemide in control animals was decreased in animals pretreated with SQ 20,881.

The existence of an intrarenal mechanism involved in stimulation of prostaglandin release has been implied from the results of a number of studies (44,45,92). The investigators of these studies have proposed that the release of interstitial renin, which is then converted to angiotensin II, stimulates a response which causes an increased synthesis of prostaglandins. Since the amount of data on lymph renin and PGE_2 secretion in the present study is not sufficient for the construction of inferences it may simply be stated that the data presented in Table I does not refute the aforementioned proposal and yet the evidence is not conclusive enough to express support.

Determination of the involvement of angiotensin II in the furosemide response was attempted in this study by observing the effects of an angiotensin II analog, saralasin, on the increase in PGE_2 secretion and renal blood flow following furosemide. Needleman et al. (77), reported that the specific competitive angiotensin antagonist, 8-cysteine angiotensin II, was capable of inhibiting the stimulation of specific renal receptor sites by angiotensin II to evoke the release of a prostaglandin-like substance. It was also shown that the angiotensin antagonist did not block the release of a prostaglandin-like substance induced by epinephrine or bradykinin.

In the present study, the increase in renal blood flow and PGE_2 secretion observed in the control group following furosemide was not significantly altered in animals treated with saralasin (Figures 7 and 8). These results suggest that furosemide acts to increase PGE_2 secretion in a manner similar to that of epinephrine and bradykinin in which the effect of the drug to increase PGE_2 secretion is not mediated by antiotensin II. It is proposed, therefore, that furosemide directly stimulates the synthesis and release of renal prostaglandins which in part is responsible for the ability of the drug to increase renal blood flow. The furosemide induced increase in renal blood flow and PGE_2 secretion observed in the saralasin group gives further support

to the proposal relating the increase in renal blood flow induced by furosemide to the corresponding increase in PGE_2 secretion (Figures 7 and 8).

In a study similar to the present, Williamson infused furosemide into the renal artery of hydropenic dogs and observed a mean increase in renal blood flow of 51 $\pm$ 6 ml/min (106). It was also shown that when these animals were pretreated with indomethacin, furosemide did not increase renal blood flow. The increase in renal blood flow observed in the volume expanded model of this study was three times as great as that increase shown in Williamson's hydropenic animals. Furthermore, when dogs in this study were pretreated with indomethacin, a significant increase in renal blood flow was still observed even though the effect of furosemide was markedly reduced (Figure 3). Interpretation of the results of these two studies suggest that the effect of volume expansion on the furosemide response is of considerable importance. This effect was evaluated when hydropenic animals receiving furosemide were compared to control animals. Furosemide did not significantly increase either renal blood flow or PGE_2 secretion in the hydropenic animals (Figures 9 and 10).

Once again the trend of renal blood flow follows that of PGE_2 secretion. While this trend may partially explain the differences in the degree of responsiveness between

these two groups, the inequality in the magnitude of diuresis may also be an important factor contributing to these differences. Furosemide produced a significantly greater increase in minute urine volume in the volume expanded than in the hydropenic animals. This profound increase in urine flow might in turn cause a significant rise in intraluminal pressure in the kidney tubules. This effect could therefore cause a condition similar to that of ureteral occlusion which has been shown to increase renal blood flow (25,52). The available evidence thus suggest that the profound diuresis induced by furosemide in volume expanded animals acted in a similar manner to that of ureteral occlusion causing in addition to the effect of increased prostaglandins an increase in renal blood flow. Partial removal of the antagonistic action of angiotensin through volume expansion may also serve in part to potentiate the effect of PGE_2 on renal blood flow following furosemide.

Because renal prostaglandins have been shown to increase urine flow and sodium excretion (40,66), it was of interest in this study to determine whether the diuresis and natriuresis induced by furosemide was mediated by a mechanism involving prostaglandins. Observations from the present study have shown that inhibition of PGE_2 secretion by indomethacin, and alteration of PGE_2 secretion following furosemide by SQ 20,881 did not significantly change the

increase in urine flow or sodium excretion induced by furosemide (Figure 11). Interpretation of these results suggest that the diruesis and natriuresis induced by furosemide is unrelated to the release of prostaglandins.

Since investigators have demonstrated that prostaglandins F and E are almost completely inactivated in cats and dogs during a single passage through the lung (22,62), it was assumed in this study that arterial PGE_2 concentration would be too low to allow for its measurement by radioimmunoassay. It was shown, however, after completion of the study, that arterial PGE_2 concentration as measured by radioimmunoassay was low, yet significant, and could therefore influence PGE_2 secretion rates. Because of this latter finding, further experiments were undertaken to determine the variability of PGE_2 concentrations measured in arterial serum under conditions similar to those encountered in the present study. While all the data concerning these experiments has not yet been evaluated, the data accumulated thus far demonstrates that values of arterial PGE_2 concentration show low variability under conditions met in this study and might therefore be treated as a constant in the derivation of renal PGE_2 secretion rates. In view of these circumstances, the reliability of data concerning PGE_2 secretion in this study is somewhat questionable and must therefore be interpreted accordingly.

SUMMARY AND CONCLUSIONS

The evidence in this study supports the following conclusions:

I. The furosemide induced increase in renal blood flow and decrease in renal resistance may in part be mediated by the potent vasodepressor, PGE_2.

II. The rise in intraluminal pressure caused by the profound diuresis of furosemide in volume expanded dogs seems to influence the degree to which furosemide induces an increase in renal blood flow and prostaglandin secretion.

III. The increase in PGE_2 secretion following furosemide demonstrates no relation to a similar increase in renin secretion.

IV. Angiotensin II shows no influence on PGE_2 secretion following furosemide.

V. The vasoconstrictive action of angiotensin II seems to be expressed following furosemide.

VI. The diuretic and natriuretic effects of furosemide are not influenced by either the renin-angiotensin system or prostaglandins.

VII. The increase in PGE_2 secretion following furosemide may arise due to the direct action of furosemide to stimulate prostaglandin synthesis, or possibly, by the action of furosemide on the metabolism of prostaglandins.

VIII. These experiments do support the theory that the renin-angiotensin system and prostaglandins influence the regulation of renal circulation, but that their mechanism of action is separate and unrelated to each other.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Aiken, J. W., and Vane, J. R. Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. J. Pharmacol. Exp. Ther. 184: 678, 1973.

2. Anderson, R. J., Berl, T., McDonald, K. M. and Schrier, R. W. Evidence for an in vivo antagonism between vasopressin and prostaglandin in the mammalian kidney. J. Clin. Invest. 56: 420-426, 1975.

3. Anggard, E., Bohman, S. O., Driffin, J. E., III, Larsson, C. and Maunsbach, A. B. Subcellular localization of the prostaglandin system in the rabbit renal papilla. Acta Physiol. Scand. 84: 231, 1972.

4. Anggard, E. The isolation and determination of prostaglandins in lungs of sheep, guines-pig, monkey and man. Biochem. Pharmacol. 14: 1507-1516, 1965.

5. Anggard, E., Larsson, C. and Samuelsson, B. The distribution of 15-hydroxy prostaglandin dehydrogenase and prostaglandin-Δ13-reductase in tissues of the swine. Acta Physiol. Scand. 81: 396, 1971.

6. Arendshorst, W. J., Johnston, P. A. and Selkurt, E. E. Effect of prostaglandin E_1 on renal hemodynamics in nondiuretic and volume-expanded dogs. Am. J. Physiol. 226: 218, 1974.

7. Attallah, A., Payakkapan, W., Lee, J., Carr, A. and Brazelton, E. PGA: Fact, not artifact. Prostaglandins 5: 69, 1974.

8. Bailie, M. D., Barbour, J. A. and Hook, J. B. Effects of indomethacin on furosemide-induced changes in renal blood flow. Proc. Soc. Exp. Biol. Med. 148: 1173-1176, 1975.

9. Belleau, L. J. and Earley, L. E. Autoregulation of renal blood flow in the presence of angiotensins in insusion. Am. J. Physiol. 213: 1590, 1976.

10. Bell, R. D., Sinclair, R. J. and Parry, W. L. The effects of indomethacin on autoregulation and the renal response to hemorrhage. Circ. Shock 2: 57, 1975.

11. Bergström, S., Danielsson, H. and Samuelsson, B. The enzymatic formation of prostaglandin E_2 from arachidonic acid. Biochem. Biophys. Acta 90: 207-210, 1964.

12. Bergström, S., Ryhage, R., Samuelsson, B. and Sjovall, J. Structure of prostaglandin E_1, F_1, and F_2. Acta Chem. Scand. 16: 501, 1962.

13. Brody, M. J. and Kadowitz, P. J. Prostaglandins as modulators of the autonomic nervous system. Fed. Proc. 33: 48, 1974.

14. Carriere, S., Friborg, J. and Guay, J. P. Vasodilators, intrarenal blood flow, and natriuresis in the dog. Am. J. Physiol. 221: 92, 1971.

15. Cornette, J. C., Harrison, K. L. and Kirton, K. T. Measurement of $PGE_{2\alpha}$ metabolites by radioimmunoassay. Prostaglandins 5: 155-164, 1974.

16. Crowshaw, K. and Szlyk, J. Z. Distribution of prostaglandins in rabbit kidney. Biochem. J. 116: 421, 1970.

17. Crowshaw, K. Prostaglandin biosynthesis from endogenous precursors in rabbit kidney. Nature, New Biol. 231: 240, 1971.

18. Daniels, E. G., Hinman, J. W., Leach, B. E. and Muirhead, E. E. Identification of prostaglandin E_2 as the principle vasodepressor lipid of rabbit renal medulla. Nature 215: 1298, 1967.

19. Davis, J. O., Carpenter, C. C. J. and Ayers, C. R. Relation of renin and angiotensin II to the control of aldosterone secretion. Circ. Res. 11: 171, 1962.

20. Dunham, E. W. and Zimmerman, B. G. Release of prostaglandin like material from dog kidney during nerve stimulation. Am. J. Physiol. 219(5): 1279-1285, 1970.

21. Euler, U. S. von and Eliasson, R. In: Prostaglandins. New York, Academic Press, 1967.

22. Ferreira, S. H. and Vane, J. R. Prostaglandins: Their disappearance from and release into the circulation. Nature 216: 868, 1967.

23. Fojas, J. E. and Schmid, H. E. Renin release, renal autoregulation, and sodium excretion in the dog. Am. J. Physiol. 219: 464, 1970.

24. Frame, M. H. and Hedqvist, P. Evidence for prostaglandin mediated prejunctional control of renal sympathetic transmitter release and vascular tone. Br. J. Pharmacol. 54: 189-196, 1975.

25. Freiman, R. H., Davis, J. O., Gotshall, R. W., Johnson, J. A. and Spielman, W. S. The signal perceived by the macula densa during changes in renin release. Circ. Res. 35: 307, 1974.

26. Freeman, R. H., Davis, J. O., Vitale, S. J. and Johnson, J. A. Intrarenal role of angiotensin II. Circ. Res. 32: 692, 1973.

27. Frolich, J. C., Sweetman, B. J., Carr, K., Splawinski, J., Watson, J. T., Anggard, E. and Oates, J. A. Occurrence of prostaglandins in human urine. Adv. Biosci. 9: 321, 1973.

28. Frolich, J. C., Wilson, T. W., Sweetman, B. J., Smigel, M., Niles, A. S., Carr, K., Watson, J. T. and Oates, J. A. Urinary prostaglandins: Identification and origin. J. Clin. Invest. 55: 763, 1975.

29. Goldblatt, M. W. Properties of human seminarl plasma. J. Physiol. (Lond.) 84: 208, 1935.

30. Goldyne, M. E., Winkelman, R. K. and Ryan, R. J. Prostaglandin activity in human cutaneous inflammation: Detection by radioimmunoassay. Prostaglandins 4: 737-749, 1973.

31. Gross, J. B. and Bartter, F. C. Effects of prostaglandins E_1, A_1 and $F_{2\alpha}$ on renal handling of salt and water. Am. J. Physiol. 225: 218, 1973.

32. Guyton, A. C., Langston, J. B. and Navar, G. Theory of renal autoregulation by feedback at the juxtaglomerular apparatus. Circ. Res. 15: I-187-197, 1964.

33. Harber, E., Koerner, T., Page, L. B., Kliman, B. and Purrode, A. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J. Clin. Endocrinol. 29: 1349-1355, 1969.

34. Hamburg, M. Biosynthesis of prostaglandins in the renal medulla of rabbit. FEBS Letters 5: 127, 1969.

35. Hamberg, M. and Samuelsson, B. The structure of the major urinary metabolite of prostaglandin E_2 in man. J. Amer. Chem. Soc. 91: 2177-2178, 1969.

36. Hedqvist, P. Antagonism by calcium of the inhibitory action of prostaglandin E_2 on sympathetic neurotransmission in the cat spleen. Acta Physiol. Scand. 80: 269, 1970.

37. Hedqvist, P., Stjarne, L. and Wennmalm, A. Inhibition by prostaglandin E_2 of sympathetic neurotransmission in the rabbit heart. ACta Physiol. Scand. 79: 139, 1970.

38. Hennam, J. F., Johnson, G. A., Newton, J. R. and Collins, W. P. Radioimmunoassay of prostaglandin $F_{2\alpha}$ in peripheral venous plasma from men and women. Prostaglandins 5: 531-542, 1972.

39. Herbaczynska, C. K. and Vane, J. R. Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in the dog. Circ. Res. 33: 428, 1973.

40. Herbaczyrska, C. K. and Vane, J. R. Prostaglandins as mediators of reactive hyperaemis in kidney. Nature 247: 429, 1974.

41. Hickler, R. B., Lauler, D. P., Sarans, C. A., Vagnucci, A. I., Stiner, G. and Thorn, G. W. Vasodepressor lipid from the renal medulla. Con. Med. Assoc. J. 90: 280-287, 1964.

42. Hofbauer, K. G., Zschiedrich, H., Rauh, W., Orth, H. and Fross, F. Reaction of endogenous renin with exogenous renin substrate within the isolated perfused rat kidney. Proc. Soc. Exp. Biol. Med. 142: 796-799, 1973.

43. Horton, E. W. Prostaglandins. In: Monographs on Endocrinology. Berlin, Heidelberg: Springer-Verlag, 1972.

44. Itskovitz, H. D., Herbert, L. A. and McGiff, J. C. Angiotensin as a possible intrarenal hormone in isolated dog kidneys. Circ. Res. 32: 550, 1973.

45. Itskovitz, H. D. and McGiff, J. C. Hormonal regulation of the renal circulation. Circ. Res. 34-35(Suppl. I): I-65, 1972.

46. Itskovitz, H. D., Stemper, J. P. D. and McGiff, J. C. Renal prostaglandins: Determinants of intrarenal distribution of blood flow in the dog. Clin. Sci. 45: 321s, 1973.

47. Jaffe, B. M., Behrman, H. R. and Parker, C. W. Radioimmunoassay measurement of prostaglandin E, A, and F in human plasma. J. Clin. Invest. 52: 398, 1973.

48. Jaffe, B. M., Smith, J. W., Newton, W. T. and Parker, C. W. Radioimmunoassay for prostaglandins. Science 171: 494, 1971.

49. Johnston, H. H., Herzog, J. P. and Lauler, D. P. Effect of prostaglandin E_1 on renal hemodynamics, sodium excretion and water excretion. Am. J. Physiol. 213(4): 939, 1967.

50. Janszen, F. H. A. and Nugteren, A. H. Histochemical localization of prostaglandin synthetase. Histochemie 27: 159, 1971.

51. Kadar, A. and Sunahara, F. A. Inhibition of prostaglandins effects by ouabain in the canine vascular tissue. Can. J. Physiol. Pharmacol. 47: 871, 1969.

52. Kalyandines, G. J., Bastron, R. A. and DiBona, G. F. Effect of ureteral clamping and increased renal arterial pressure on renin release. Am. J. Physiol. 225: 95, 1973.

53. Keirse, M. J. N. C. and Turnbull, A. C. Gas chromatographic determinations of E prostaglandins in human amniotic fluid. Prostaglandins 4: 263-268, 1973.

54. Kilcoyne, M. M. and Cannon, P. J. Influence of thoracic caval occlusion on intrarenal blood flow distribution and sodium excretion. Am. J. Physiol. 220: 1220, 1971.

55. Kotchin, T. A. and Miller, M. C. Effect of prostaglandin on renin activity. Am. J. Physiol. 226: 314, 1974.

56. Kurzrok, R. and Lieb, C. C. Proc. Soc. Exp. Biol. Med. 28: 268-272, 1930.

57. Larsson, C. and Anggard, E. Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. Eur. J. Pharmacol. 21: 30, 1973.

58. Lee, J. B., Crowshaw, K., Takman, B. H. and Attrep, K. A. The identification of prostaglandins E_2, $F_{2\alpha}$, and A_2 from rabbit kidney medulla. Biochem. J. 105: 1251, 1967.

59. Lee, J. B., Convino, B. G., Takman, B. H. and Smith, E. R. Renomedullary vasodepressor substance, medullin: Isolation, chemical characterization and physiological properties. Circ. Res. 17: 57, 1965.

60. Leyssac, P. P. Intrarenal function of angiotensin. Fed. Proc. 26: 55-59, 1967.

61. Lonigro, A. J., Itskovitz, H. A., Crowshaw, K. and McGiff, J. C. Dependency of renal blood flow on prostaglandins synthesis in the dog. Circ. Res. 32: 712, 1973.

62. Louis, W. J. and Doyle, A. E. Effects of varying doses of angiotensin on renal function and blood pressure in man and dogs. Clin. Sci. 29: 489, 1965.

63. Martinez-Maldonado, M., Tsaparas, N., Eknoyan, G. and Suki, W. W. Renal actions of prostaglandins: Comparison with acetylcholine and volume expansion. Am. J. Physiol. 222: 1147, 1972.

64. McGiff, J. C., Crowshaw, K., Terragno, N. A. and Lonigro, A. J. Mechanisms of hypertension. Circ. Res. (Suppl. I): I-121, 1970.

65. McGiff, J. C., Crowshaw, K., Terragno, N. A., Lonigro, A. J., Strand, J. C., Williamson, N. A., Lee, J. B. and Ng, K. K. F. Prostaglandin-like substances appearing in canine renal venous blood during renal ischemia. Circ. Res. 27: 765-782, 1970.

66. McGiff, J. C., Crowshaw, K., Terragno, N. A. and Lonigro, A. J. Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. Circ. Res. 27(Suppl. I): 121, 1970.

67. McGiff, J. C., Itskovitz, H. D. and Crowshaw, K. Prostaglandins and renal function. Fed. Proc. 3: 39, 1974.

68. McGiff, J. C., Terragno, N. A., Malik, K. V. and Lonigro, A. J. Release of a prostaglandin E-like substance from canine kidney by bradykinin. Circ. Res. 31: 36, 1972.

69. McGiff, J. A., Terragno, N. A., Strand, J. C., Lee, J. B. and Lonigro, A. J. Selective passage of prostaglandins across the lung. Nature 223: 442, 1969.

70. McNay, J. L. and Abe, Y. Pressure-dependent heterogeneity of renal cortical blood flow in dogs. Circ. Res. 27: 571, 1970.

71. Middleditch, B. S. and Desiderio, D. M. Gas chromatography of prostaglandin heptaflurobutyrate methyl esters. Prostaglandins 2: 195-198, 1972.

72. Middleditch, B. S. and Desiderio, D. M. Gas-liquid chromatography of trimethylsilyl and alkyl oximetrimethylsilyly derivatives of some prostaglandins. Prostaglandins 2: 115-121, 1972.

73. Mimram, A., Casellas, D., Dupont, M. and Barjon, P. Effects of a competitive angiotensin antagonist on the renal hemodynamic changes induced by inhibition of prostaglandin synthesis in rats. Clin. Sci. Mol. Med. 48: 299s, 1975.

74. Muirhead, E. E., Germain, G. S., Leach, B. E., Brooks, B. and Stephenson, P. Renomedullary interstitial cells (RIC), prostaglandins (PG), and the hypertension function of the kidney. Prostaglandins 3: 581, 1973.

75. Muirhead, E. E., Germain, G. S., Leach, B. E., Pitcock, J. A., Stephenson, P., Brooks, B., Brosius, W. L., Daniels, E. G. and Hinman, J. W. Production of renomedullary prostaglandins by renomedullary interstitial cells grown in tissue culture. Circ. Res. 31(Suppl. II): 161, 1972.

76. Mulrow, P. J. and Ganong, W. F. Stimulation of aldosterone secretion by angiotensin II. Yale J. Biol. and Med. 33: 386, 1961.

77. Needleman, P., Kauffman, A. H., Douglas, J. R., Jr., Johnson, E. M., Jr., and Marshall, G. R. Specific stimulation and inhibition of renal prostaglandin release by angiotensin analogs. Amer. J. Physiol. 224: 1415-1419, 1973.

78. Neddleman, P., Douglas, J. R., Jr., Sakschik, B., Stoeckfein, P. B. and Johnson, E. M., Jr. Release of renal prostaglandins by catecholamines: Relationship to renal endocrine function. J. Pharmacol. Exp. Ther. 188: 453-460, 1974.

79. Nissen, H. M. and Andersen, H. On the localization of a prostaglandin dehydrogenase activity in the kidney. Histochemie 14: 189, 1968.

80. Owen, T. L., Ehrhart, I. C., Weidner, W. J., Scott, J. B. and Haddy, F. S. Effects of indomethacin on local blood flow regulation in canine heart and kidney. Fed. Proc. 33: 348, 1974.

81. Paulsrud, J. R. and Miller, O. N. Inhibition of 15-OH prostaglandin dehydrogenase by several diuretics. Fed. Proc. 33: 590, 1974.

82. Prezyna, A., Attallah, A., Vance, K., Schoolman, M. and Lee, J. A newly recognized structure of renomedullary interstitial cell origin associated with high prostaglandin content. Prostaglandin 3: 669, 1973.

83. Rector, J. B., Stein, J. H., Bay, W. H., Osgood, R. W. and Ferris, T. F. Effect of hemorrhage and vasopressor agents on distribution of renal blood flow. Am. J. Physiol. 222: 1125, 1972.

84. Satoh, S. and Zimmerman, B. G. Participation of intrarenal prostaglandin in renal ischemia. Fed. Proc. 33: 576, 1974.

85. Schmid, H. E. Renal autoregulation and renin release during changes in renal perfusion pressure. Am. J. Physiol. 222: 1132, 1972.

86. Scnmid, H. E. Renin, a physiologic regulator of renal hemodynamics? Circ. Res. 11: 185-193, 1962.

87. Sirois, P. and Gagnon, D. J. Release of prostaglandins from the rabbit renal medulla. Eur. J. Pharmacol. 28: 18-24, 1974.

88. Sokal, R. R. and Rohl, F. J. In: Biometry. San Francisco, California, W. H. Freeman and Co., 1969.

89. Somova, L. A study on the vasodepressor lipid isolated from kidneys of hypertensive animals. Nephron 8: 575, 1971.

90. Strong, C. G., Boucher, R., Nowaczynski, W. and Gehest, J. Renal vasodepressor lipid. Proc. Staff Meetings Mayo Clinic 41: 433-452, 1966.

91. Stygles, V. G., Rothenberg, R. J., Bailie, M. D., Hook, J. B. and Reinke, D. A. Preparation of an antibody specific for PGE_2 using the thyroglobulin as the hapten. Personal communication.

92. Sacumu, S. and Zimmerman, B. G. Influence of the renin angiotensin system on the effect of prostaglandin synthetase inhibitors in the renal vasculature. Circ. Res. 36-37(Suppl. I): I-89, 1975.

93. Tannenbaum, J., Splawinski, J. A., Oates, J. A. and Nies, A. S. Enhanced renal prostaglandin production in the dog. Circ. Res. 36: 204, 1975.

94. Thompson, C. J., Los, M. and Horton, E. W. Separation, identification and estimation of prostaglandins in nanogram quantities by combined gas chromatography-mass spectrometry. Life Sci. 9: 983-988, 1970.

95. Thurau, K. Renal hemodynamics. Am. J. Med. 36: 686-719, 1964.

96. Tobian, L., O'Donnell, M. and Smith, P. Intrarenal prostaglandin levels during normal and high sodium intake. Circ. Res. 34 and 35 (Suppl. I): I-83, 1974.

97. Vander, A. J. Direct effects of prostaglandin on renal function and renin release in anesthetized dogs. Am. J. Physiol. 214(2): 218-221, 1968.

98. Vander, A. J. and Carlson, J. Mechanism of the effects of furosemide on renin secretion in anesthetized dogs. Circ. Res. 25: 145, 1969.

99. Van Dorp, D. A., Beerthuis, R. K., Nugteren, D. H. and Vonkeman, H. The biosynthesis of prostaglandins. Biochem. Biophys. Acta 90: 204-207, 1964.

100. Vane, J. R. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature, New Biol. 231: 232, 1971.

101. Vane, J. R. The release and fate of vasoactive hormones in the circulation. Br. J. Pharmacol. 35: 209-242, 1969.

102. Walser, M., Davidson, D. G. and Orloff, J. The renal clearance of alkali-stable inulin. J. Clin. Invest. 34: 1520-1523, 1955.

103. Weiner, R. and Kaley, G. Influence of prostaglandin E on the terminal vascular bed. Am. J. Physiol. 217: 536, 1969.

104. Werning, C., Vetter, W., Weidmann, P., Schweiker, H. V., Stiel, D. and Siegenthaler, W. Effects of prostaglandin E_1 on renin in the dog. Am. J. Physiol. 220: 852, 1971.

105. Williams, M. W., Wilson, T. W., Oates, J. A., Niles, A. S. and Frolick, J. C. Origin of urinary prostaglandins. J. Clin. Invest. 53: 85a, 1974.

106. Williamson, H. E., Bourland, W. A. and Marchand, G. R. Inhibitions of furosemide induced increase in renal blood flow indomethacin. Proc. Soc. Exp. Biol. Med. 148: 164, 1975.

107. Zusman, R. M., Caldwell, B. V. and Speroff, L. Radioimmunoassay of the A prostaglandins. Prostaglandins 2: 41, 1972.

108. Zusman, R. M., Snider, J. J., Cline, A., Caldwell, B. V. and Speroff, L. Antihypertensive function of renal cell carcinoma. New Engl. J. Med. 290: 843, 1974.

109. Zusman, R. M., Spector, D., Caldwell, B. V., Speroff, L., Borman, B., Schneider, G. and Mulrow, P. Prostaglandin A concentrations in plasma of normal and hypertensive humans. J. Clin. Invest. 52: 93a, 1973.