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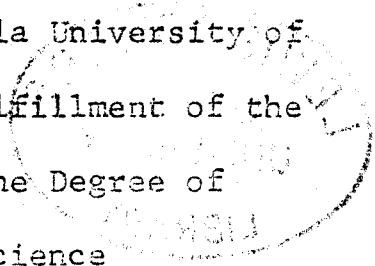
IN VITRO RENIN SECRETORY RESPONSES TO

PGE$_2$, PGF$_2$-ALPHA, INDOMETHACIN AND

ADRENERGIC-RECEPTOR BLOCKER ADMINISTRATION

by

Brett C. Sheppard

A Thesis Submitted to the Faculty of the

Graduate School of Loyola University of

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VITA

The author, Brett C. Sheppard, is the son of Neil N. Sheppard and Rosalyn (Constantine) Sheppard. He was born February 21, 1956, in Harvey, Illinois.

His elementary education was obtained in the public school system of Flossmoor, Illinois, and secondary education at the Homewood-Flossmoor High School, Flossmoor, Illinois, where he graduated in June, 1974.

In September of 1974 he entered the Michigan State University (East Lansing, MI). In August 1975 he transferred to the University of Wisconsin-Madison and in August, 1978, received the degree of Bachelor of Sciences with a major in Zoology.

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Pharmacol., 1980; Lopez, Rao, Mottel and Sheppard, Experientia, 1980), as well as co-author of papers presented at regional (Illinois Acad. Sci., Lisle, IL., 1980) and national meetings (Fed. Am. Soc. Exp. Biol. Med., Anaheim, CA, 1980).

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INTRODUCTION

Data generated in this laboratory (1,2) utilizing a renal cortical slice preparation from sodium deficient rats, suggest that dopamine directly stimulates renin release via a beta-adrenoreceptor mechanism operating at the level of the juxtaglomerular cell membrane. This effect appears to be coupled with increases in the concentration of cyclic 3':5'-adenosine monophosphate (c-AMP) in the renal cortical tissue, suggesting the participation of this nucleotide as the intracellular mediator of the stimulatory effect of dopamine on renin secretion. Recently (3), we have further supported this concept by demonstrating that addition of the phosphodiesterase inhibitor, theophylline, to the kidney slice system, potentiates the renin release and c-AMP responses to exogenous dopamine administration. Additionally, we have proposed (4) that dopamine may inhibit renin release through a dopamine-specific receptor as opposed to its beta-adrenoreceptor mediated stimulatory effect, suggesting a dual regulatory role for dopamine in the overall regulation of renin release.

Intrarenal prostaglandins (PGs) have also been implicated in the control of renin release (5,6). _In_ _vivo_ (7,8, 213,214) and _in_ _vitro_ (9,10) observations suggest that exogenous prostaglandin E_2 (PGE_2) stimulates renin secretion, but in several instances (11-14) this agent was found to be ineffective in altering the release of renin. Alternatively, prostaglandin F_2-alpha (PGF_2-alpha) has been shown to either increase (10) or decrease renin release _in_ _vitro_ (9,13) or to have no effect _in_ _vivo_ (15).

Renal synthesis of prostaglandins occurs mostly in the medullary areas (16,17), but some prostaglandins can also be produced in the renal cortex (18,19). Renal cortical prostaglandin synthesis has been shown to be stimulated by arachionic acid (C20:4) administration (20,21), and to be inhibited by the effective prostaglandin synthetase blocker, indomethacin (22,23,215), as well as by other anti-inflammatory agents (24,25). Although the mechanism by which intrarenal prostaglandins influence renin secretion may be related to their effects on the renal vasculature (26,27,216), recent _in_ _vivo_ data have suggested that they may affect renin release via a direct action on the juxtaglomerular cells (28).

In *vitro* renal cortical preparations are particularly suitable for an effective evaluation of factors which may directly influence renin secretion. The release of renin from the juxtaglomerular cells is influenced by a number of hemodynamic and humoral factors (29,30) and by the nervous system (31,32). An *in vitro* renal cortical system, by eliminating these influences, thus allows for the examination of a direct action of prostaglandins and other agents on renin release. Furthermore, *in vivo* (33,34) and *in vitro* (1,2,35,36, 37) evidence suggests that dietary sodium deficiency not only increases resting renin release, but that it also potentiates the renin secretory rate elicited by sympathoadrenergic discharge. Recent *in vitro* studies (38,39,40) have additionally demonstrated that dietary sodium deficiency also potentiates the resting concentration and release of intrarenal prostaglandins. Thus, a renal cortical slice preparation from sodium deficient rats which maximizes renin release and possibly intrarenal prostaglandin concentration as well, is a viable model for investigating the mechanism of action by which intrarenal prostaglandins may influence renin release.

Therefore, in view of the conflicting nature of the data regarding renal prostaglandin mediation of renin, this study

has been designed to (1) determine if exogenously administered prostaglandins of the E and F series can directly influence renin release in a renal cortical slice system from sodium deficient rats, (2) investigate if blockade of renal alpha- and beta-adrenergic receptors modifies the renin secretory responses to prostaglandin administration, (3) examine if changes in renal cortical tissue c-AMP content in response to prostaglandin treatment are coupled with those seen in renin release, and (4) evaluate if administration of indomethacin, an effective prostaglandin synthetase inhibitor, can influence the increased resting renin secretory levels seen in sodium deficient rats. A positive finding would support the concept that intrarenal prostaglandins participate in the regulation of resting renin release in the sodium deficient state.

LITERATURE REVIEW

I. Introduction to the Renin-Angiotensin System. (RAS)

Since the discovery of renin by Tigerstedt (41) numer-
ous studies have clearly implicated the RAS in the regulation
of blood pressure, primarily via the pressor action of Angio-
tensin II (ANG II) on peripheral blood vessels. The ANG II
octapeptide is synthesized via the catalytic action of renin.
In turn, renin cleaves angiotensinogen of hepatic origin to
form the decapeptide Angiotensin I (ANG I), the immediate
precursor of Angiotensin II. ANG I is in turn hydrolyzed by
converting enzyme, present largely in the pulmonary vascular
bed, to ANG II. Subsequent proteolytic action of circulating
angiotensinases results in the formation of Angiotensin III
(ANG III), a peptide with little pressor activity, and inac-
tive metabolites (30). The RAS has also been shown to func-
tion in the regulation of blood volume and electrolyte homeo-
stasis. ANG II has been demonstrated to influence sodium
excretion through its effect on the renal vasculature which
alters the filtered sodium load and the sum of Starling
forces acting on the pertibular capillaries (42). Additional-
ly, ANG II infusions stimulate the synthesis and release of

aldosterone and vasopressin (43,44,217), which are of primary importance in renal sodium and water reabsorption.

II. Anatomical Evidence.

The current accepted view places the site of formation, storage and release of renin in the juxtaglomerular cells (JG) of the afferent arteriole of the mammalian kidney, although compounds with renin-like activity have been demonstrated in extrarenal regions as well (45,46). Granular cells lining the afferent arteriole wall closest to the glomerulus were first reported by Oberling and Hatt (47) and later confirmed by other investigators (48,49,218,219,220), and sodium deficiency has been shown to increase the number and granulation of rat JG cells (53,37).

III. Regulatory Mechanisms Involved in the Control of Renin Secretion.

A. The Macula Densa Receptor.

Substantial evidence suggests the existence of a functional relationship between the macula densa and the granular cells involved in renin secretion. However, the exact nature of the signal detected by the cells of the macula densa and the mechanism by which the signal is detected and transmitted to the

renin-secreting cells is still open to question.
Barajas (48), based on electron micrographs of rat
JG cells, has suggested that decreased contact be-
tween these cells and the macula densa cells of the
distal tubule would result in increased renin secre-
tion. This decreased stretch of the tubular cells
has been attributed to a lowered sodium load in the
distal tubule with an accompanying volume reduction,
thus resulting in decreased contact.

Micropuncture experiments (50) point to an in-
verse relationship between renin release and total
sodium load delivered to the macula densa. This in-
verse relationship has been confirmed by Nash et al.
(51) who demonstrated in dogs the dissociation of
renin release from renal hemodynamics and water and
sodium balance, but not from the filtered load of
sodium. They suggested the existence of an intra-
renal, extravascular sodium-sensitive mechanism,
which responds directly to changes in sodium flux
across the macula densa. Recent work in the isolated
rat kidney has lent support to this concept (52).
However, studies utilizing retrograde injections of

high sodium buffer into the distal tubule have suggested instead a direct relationship between filtered sodium load and renin release (53,221).

B. The Renal Baroreceptor.

Embryonically, JG cells are derived from stem cells which normally form smooth muscle (54), although they have developed the ability to functionally behave as endocrine cells, which the remaining smooth muscle cells of the afferent arteriole do not have. The latter, however, can indirectly affect JG cell function by responding to changes in the stretch of their walls. Therefore, the existence of a renal vascular receptor, sensitive to stretch and thus to alterations in renal perfusion pressure has been investigated. In denervated-, adrenalectomized-, non-filtering canine kidneys, renin secretion has been observed to increase in response to decreased renal perfusion pressure associated with hemorrhage and graded suprarenal aortic constriction (55,56,57,222). This increased renin secretory response was no longer apparent when perfusion pressures were restored to normal levels. Furthermore, _in vivo_ administration of the

smooth muscle relaxant papaverine has been demonstrated to abolish the renin secretory responses to hemorrhage in canine-denervated-, non-filtering kidneys (58), although it is possible that this effect may have been due to a direct effect of papaverine on the JG cells. Recent in vitro experiments utilizing a rat renal cortical cell suspension have shown papaverine may directly inhibit renin secretion (59).

C. The Nervous System.

1. Central Nervous System Regulation of Renin Release.

The possibility of a brain renin-angiotensin system participating in the central regulation of peripheral blood pressure has long been debated. There is generalized agreement that circulating ANG II or ANG III influence some brain regions located outside the blood-brain barrier (60). Several components of the RAS have been demonstrated intracerebrally as well (61). However, the issue of whether it is renin or an isorenin which is present intracerebrally remains unresolved. Endogenous brain renin and the renin-like enzyme, Cathepsin D, co-purify by gel filtration and ammonium sulphate fractionation (62); Cathepsin D, as

does renin, will react with renin substrate to form ANG I (63), although the activity of this so-called "brain-renin" decreases above pH 7.0, while the optimal pH for renal renin activity is at 7.4 (64). Recent investigations have demonstrated a fraction of Cathepsin D which has renin-like optional activity at pH 7.4 and is effectively neutralized by anti-renin anti-sera (65,66,67, 223,224,225).

a. The Effect of Plasma Angiotensin on the Brain.

Confirmation of plasma angiotensin influence on the brain was provided in an elegant experiment by Bickerton et al. (68). In this cross-perfusion study, the head of a recipient dog, isolated from its circulation but connected to its spinal cord, was perfused with blood from a donor dog receiving ANG II injected into its circulation. Peripheral arterial pressure was observed to increase in the recipient dog while ANG II was infused into the donor.

Mediation of plasma ANG II influence on the CNS must occur outside the blood-brain

barrier, since this peptide cannot cross the blood-brain barrier (69). The pressor response to ANG II infusions into the vertebral artery of dogs has been shown to occur only if the circulation to the area postrema, outside the blood-brain barrier, remains unimpaired (70). Moreover, injections of ANG II into the vertebral artery inhibit reflex vasodilation in the dog (70), suggesting a possible role for ANG II in modifying the inhibitory activity exerted by vasomotor effector neurons (70). Neural connections have also been demonstrated between the area postrema and the nucleus of the tractus solitarius (70) which receives input from the glossopharyngeal and carotoid sinus nerves and in turn operate as a relay for afferent baroreceptor impulse integration (71).

Direct administration of ANG II into the cerebral ventricles causes increased secretion of both vasopressin and ACTH and a rise in blood pressure (44,72). These

responses have been mimicked by intraventricular injection of purified renin (73), and responses to both peptides are abolished by the competitive ANG II antagonist, Saralsin (73). Additionally, converting enzyme inhibitors have been shown to block the response to intracerebral injection of renin (73), thus further supporting the view that the observed responses are due to ANG II of cerebral, rather than peripheral origin.

b. Central Influence on Renal Renin Release.

The postulation that the CNS plays a role in modulating renal renin release has been arrived at as a result of the following observations: (1) Cathepsin D, an enzyme apparently containing a renin-like component, activates inactive renin in human amniotic fluid (74). This observation taken together with the intracerebral demonstration of Cathepsin D suggests a possible central physiological role for this enzyme; (2) central mediation of peripheral activation of inactive renin has been shown (75); (3) in

vivo pharmacological evidence indicates that stimulation of central serotoninergic pathways increases peripheral renin release (76); (4) stimulation of the posterior hypothalamus, medulla oblongata, and pons, increases renin secretion and is associated with a rise in blood pressure; and (5) renal denervation abolishes the increase in renin release in response to central stimulation (73).

Clonidine, a known central alpha-receptor agonist agent, has several actions on the brain. It decreases plasma ACTH through an action on the hypothalamus, and injections of this agent into the blood perfusing the medulla and the pons result in a significant depressor response (73). Furthermore, vertebral or carotoid arterial administration of clonidine produces a decrease in plasma renin activity (73). Bilateral lesions of the canine ventral medulla abolish the decreased splanchnic nerve discharge associated with clonidine injection, while the depressor response remains

intact. Thus, these data point to a separate site for the renin-lowering effect and the depressor responses to this agent (73).

Injections of clonidine into the cisterna magna of anesthetized, bilaterally splanchnictomized dogs, do not result in decreased plasma renin activity, whereas the same dose injected into animals with intact splanchnic nerves result in a significant decrease in hemorrhage-stimulated increases in plasma renin activity. These data suggest that clonidine reduces sympathetically-stimulated renin via a central mechanism dependent on splanchnic nerve integrity (77). The renin-lowering effect of clonidine may be exerted through an excitatory action on intracerebral alpha-adrenoreceptors (73). Clonidine has been shown to have a greater affinity for pre- rather than post-junctional alpha-adrenoreceptors (78). However, the evidence regarding the action of clonidine on renin release is not yet complete. Clonidine appears to decrease sympathetic discharge rate

even when pre-synaptic inhibition is abolished
by reserpine-induced catecholamine depletion
(79). Additionally, a neurally-mediated
decrease in renin secretion, which can be
abolished by renal denervation, has also been
reported when L-dopa is administered with car-
bidopa to provoke catecholamine release (80).
Furthermore, while injections of norepineph-
rine, epinephrine and dopamine into the third
ventricle of the dog increase renin secretion,
injections of clonidine into the same site
decrease renin secretion (73). Thus, these
data indicate that agents with high affini-
ties for post-synaptic receptors increase
renin secretion while agents with high affini-
ties for pre-synaptic receptors decrease
renin secretion.

2. Peripheral Nervous System Regulation of Renin
Release.

a. Anatomical and Functional Evidence.

A large body of evidence suggests that
the sympathetic nervous system innervates the
JG cells and thus can directly modulate renin

release. Electron microscopic studies (81-83) have clearly shown this extensive sympathetic innervation of the renin-secreting cells, and fluorescent histochemical studies and chemical assays (84,85) have further supported this view. Furthermore, circulating catecholamines of adrenal medullary origin can directly influence renin release by acting on renal adrenergic receptors, in addition to their indirect effects exerted through the renal baroreceptors and macula densa receptors (29-32).

Functional evidence for a direct sympathetic effect on renin secretion, independent of hemodynamic and macula densa influences, has been provided by numerous studies. In papaverine-treated dogs with non-filtering kidneys, electrical stimulation of the renal nerves increases renin release (86). Similarly, in vitro administration of catecholamines stimulates renin secretion (1,2,35,36,87,88, 96,98), presumably by a direct action on the JG cells.

b. The Renal Beta-Adrenergic Receptor.

Abundant in vivo and in vitro data have firmly established the concept that circulating catecholamines and norephinephrine released by renal nerve terminals, can directly stimulate renin release (1,2,89-93). This direct stimulatory effect appears to be exerted via a JG cell membrane-bound beta-adreno-receptor mechanism (36,90,94,96,100-102,104, 226), since blockade of this receptor mechanism by specific pharmacological agents such as propranolol and pindolol effectively prevents the increase in renin release seen in response to sympathetic stimulation (31,36,90,92,94-97, 100-104,227,228).

The stimulatory action of beta-adreno-receptor activation on renin secretion has been suggested to be coupled with a simultaneous increase in the levels of cyclic 3!5'-adenosine monophosphate (c-AMP) in renal tissue (1,2,36) which may indicate that this nucleotide acts as the intracellular mediator of sympathetic stimulation of renin release

(229). This view has been further supported by the demonstration that the phosphodiesterase inhibitor, theophylline, potentiates the stimulatory action of catecholamines on renin release (98,99,105,107). Additionally, administration of dibutyryl c-AMP to isolated rat renal cortical tissue increases renin release in a dose-dependent fashion (108), an observation which has been confirmed in other in vivo and in vitro studies (90,106,109).

The type of beta-adrenoreceptor specifically involved in the regulation of renin release has not yet been fully determined. Recent data suggest the involvement of a $beta_1$-adrenoreceptor rather than a $beta_2$-type similar to the one present in cardiac tissue (110-112, 230), but some in vitro studies indicate that the renal beta-adrenoreceptor may not belong to either type (113). Furthermore, Saruta et al. (114), have suggested that the decrease in renin release associated with beta-adrenergic blockade is due to a membrane stabilizing

effect of the blocking agents rather than to their functional beta-blocking actions.

c. <u>The Renal Alpha-Adrenergic Receptor</u>.

Stimulation of intrarenal alpha-adreno-receptors has been shown to result in an inhibition of renin release (100,115-117). Furthermore, alpha-adrenoreceptor antagonists have been demonstrated to abolish the inhibitory effect of alpha-adrenoreceptor activation of renin release (36,93,98,100,118). Taken together, these observations suggest an inhibitory role for intrarenal alpha-adreno-receptors in the regulation of renin release.

d. <u>The Renal Dopamine Receptor</u>.

Dopamine has been shown to be present in canine renal tissue (118). The renal vaso-dilating properties of this amine have been postulated to indirectly influence renin release (120,121,122), and these renal vascular effects apparently involve an interaction with dopamine-specific receptors located on the renal vascular walls (123,124,125).

Dopamine has also been found to increase the c-AMP content of rat renal particulate preparations (126,127) which suggest that the renal vascular actions of this amine may be mediated by adenylate cyclase systems functionally coupled with renal vascular dopaminergic receptors. Alternatively, the observed changes in renal tissue c-AMP content in response to dopamine administration may reflect an action of this agent on cells other than vascular cells, such as the JG cells, suggesting adenylate cyclase mediation of a direct effect of dopamine on renin release (128,129). This possibility has now been suggested by in vitro studies (1-3,130,131), in which dopamine administration simultaneously stimulated both renin release and tissue c-AMP content in rat renal cortical tissue. Furthermore, this effect was shown to be effectively blocked by propranolol in these studies, suggesting that mediation of the stimulatory action of dopamine on renin

secretion is via a beta-adrenoreceptor mechanism. Conversely, administration of dopamine-receptor blockers such as haloperidol (131) and d-butaclamol (4) has been demonstrated to potentiate the stimulation of renin release by dopamine, which may indicate that renal dopaminergic receptors may regulate renin release in an inhibitory fashion. The physiological significance of this dual regulation is unclear, but a similar dual type of control has been proposed to exist in regard to the action of norepinephrine on renin release (36,115,117,118).

3. Humoral Influences in the Control of Renin Release.

a. Angiotensin II (ANG II).

Peripheral infusions of ANG II have been shown to inhibit renin secretion (42). In sodium deficient sheep (132), chronic ANG II administration prevented the expected rise in plasma renin activity, seen in the sodium deficient state. ANG II has also been reported to inhibit renin secretion in the absence of a functional renal baroreceptor or macula densa (133), suggesting a direct inhibitory action of ANG II on the JG cells. Furthermore, Keeton

et al. (134) have suggested that infusions of
saralasin, an ANG II competitive inhibitor,
disrupts the ANG II short-loop, local inhibi-
tory feedback mechanism on renin release,
which is also associated with the intrarenal
beta-adrenoreceptors, and in vitro observa-
tions by Ganong and Lopez (36) have demonstrat-
ed that ANG II administration inhibits the
stimulatory effect of norepinephrine on renin
secretion, and that this effect is coupled
with corresponding decreases in the c-AMP con-
tent of the renal cortical tissue. Thus, it
appears that in addition to its indirect ac-
tions in the regulation of renin release,
namely, via its central and peripheral vascu-
lar effects, ANG II has the ability to locally
control the secretion on renin by a direct
short-loop feedback mechanism operating at the
JG cell level. However, a negative feedback
effect exerted by ANG II on the JG cells was
not observed in a recent in vivo investigation
by Baer and her associates (135).

b. Vasopressin (ADH).

Some investigators have also reported
that ANG II infusions increase the plasma
concentration of vasopressin (44,136,137).
Since it has also been shown that vasopres-
sin inhibits renin release (44,137,138), it
may be possible that the inhibitory regula-
tion of renin release by vasopressin may be
the result of ANG II-mediated central stimu-
lation of vasopressin release, although the
actual mechanism by which vasopressin in-
fluences renin secretion remains unclear.

c. Electrolytes.

In addition to sodium, other electrolytes
also appear to regulate renin secretion. Chlo-
rine has recently been reported to increase
renin release in rats independent of sodium
concentration (139). In experiments with de-
nervated, auto-transplanted canine kidneys, hy-
permagnesia has been demonstrated to stimu-
late renin release independent of hemodynamic,
nervous and natriuretic factors (140). The
mechanism of action involved in this effect

appears to be related to the antagonism which exists between magnesium and calcium in controlling renin release. Recent in vitro work (141) has demonstrated that potassium-free media causes a depression of renin release in renal cortical tissue, and that this effect is partially prevented when calcium is also absent from the media. Vandongen and Peart (142) have also demonstrated that ANG II will not inhibit basal renin secretion in the absence of calcium. These observations point to a regulatory influence of calcium on a JG cell membrane response to ionic influences (231,232).

4. <u>Regulation of Renin Secretion by Intrarenal Prostaglandins</u>.

Synthesis of prostaglandins (PG) has been demonstrated to occur in a variety of peripheral vascular beds (143,144). In the kidney, the concentration of PG synthetase is highest in the medulla and papillae but there is also significant PG production in the renal cortex (17-20,145-147). Renal cortical synthesis of prostaglandins is

dependent on the conversion of free arachidonic acid (C20:4) to the endoperoxide PGG_2 via the action of cyclooxygenases. Subsequent reductase activity results in the biosynthesis of PGE_2 or PGF_2-alpha while isomerases catalyze the conversion of PGG_2 or PGF_2. Additionally, PGE_2 may be converted to PFG_2-alpha via the action of PGE 9-ketoreductases (148). Although it is generally accepted that renal prostaglandins play a predominant role in the control of renal vascular dynamics (25,26,144), their presence in the renal cortex has led to numerous studies designed to investigate the possibility of a regulatory influence of these agents on renin secretion (5,6, 149-155), either directly or indirectly.

a. <u>Indirect Regulation of Renin Secretion by Prostaglandins</u>.

    i. <u>PG Involvement in the Macula Densa and Baroreceptor Control Mechanisms</u>.

        Prostaglandins appear to be required for effective macula densa function. In unilaterally renal denervated-, contra-laterally nephrectomized-, papaverine- and

propranolol-treated anesthetized dogs, the PG synthetase inhibitor indomethacin, has been shown to abolish aortic clamp-stimulated increases in plasma renin activity (156). Additionally, indomethacin can inhibit the increased plasma renin activity associated with decreased early distal tubular sodium load in both sodium-replete and deficient rats (157).

Studies utilizing adrenalectomized-, renal denervated-, propranolol-treated, non-filtering canine kidneys with contra-lateral nephrectomy, have suggested a role for cortical PGs in the intrarenal baro-receptor control of renin release (158). In this study, indomethacin abolished the rise in plasma renin activity associated with decreased renal perfusion pressure and intrarenal infusion of $C20:4$. This suggests that cortical, rather than medul-lary PGs are involved in the intrarenal baroreceptor response, since medullary cyclooxygenase products cannot be

transported to the cortex via tubular
fluid in treated anesthetized dogs, the
PG synthetase inhibitor indomethacin, has
been shown to abolish aortic clamp-
stimulated increases in plasma renin
activity (156). Additionally, indometha-
cin can inhibit the increased plasma
renin activity associated with decreased
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(158). In this study, indomethacin abol-
ished the rise in plasma renin activity
associated with decreased renal perfusion
pressure and intrarenal infusion of C20:4.
This suggests that cortical, rather than
medullary PGs are involved in the intra-
renal baroreceptor response, since medullary

cyclooxygenase products cannot be transported to the cortex via tubular fluid in the non-filtering kidney (158). These observations have been recently extended by the demonstration that PGE_2 and PGI_2 of cortical origin are necessary for baroreceptor stimulated renin release (159). Thus, current evidence suggests the involvement of medullary and cortical PGs in two of the three major mechanisms which regulate renin release.

ii. The Relationship Between PGs and Angiotensin II (ANG II).

In vivo evidence has indicated endogenous renal PG mediation of the ANG II short-loop feedback mechanism. Administration of the ANG I converting enzyme inhibitor, SQ 14225, results in a significant increase in plasma renin activity which is abolished following indomethacin infusion (160). Indomethacin has also been shown to decrease ANG II-induced aldosterone release in sodium deficient

rats (161). Alternatively, experiments in the isolated rat kidney suggest that ANG II increases PGE_2 biosynthesis (162), and the production of an increased renal venous PGE-like agent associated with low frequency renal nerve stimulation, has also been reported to be mediated by ANG II (163).

iii. <u>PGs and the Renal Nerves</u>.

While experimental evidence suggests a role for PGs in modulating sympathetically-evoked renin release, the mechanism involved in this action has not yet been determined (164,165). In renal-denervated dogs, treated with cyclooxygenase inhibitors while undergoing hemorrhage, plasma renin activity remained unaltered in relation to control levels (166). However, direct stimulation of the renal sympathetic nerves in anesthetized dogs has been shown to simultaneously increase renin release and PGE_2 levels two-fold over those of control (167). Furthermore, isoproternol-stimulated renin release in rats is reduced by 67% following administration of

indomethacin, while this same agent attenu-
ated the rise in serum renin levels caused
by the $beta_1$-adrenoreceptor agonist H133/22
by 80% (168). Catecholamine infusions have
also been reported to increase PG release
in the isolated, perfused rabbit kidney,
thus suggesting a role for the renal
nerves in stimulating PG synthesis (169).
These observations taken together, support
the existence of a mechanism whereby sympa-
thetic stimulation, which may be under the
tonic restraint of cardiovascular reflexes,
evokes simultaneous increases in renin re-
lease and PG biosynthesis, with PGs act-
ing at a site distal to the intrarenal
beta-adrenergic receptor.

iv. PG Synthetase Inhibitors and Their Relation
to the RAS.

In an attempt to clarify the mechanism
by which intrarenal PGs may influence renin
release, the non-selective, cyclooxygenase
inhibitor, indomethacin, has been utilized
in a number of investigations. Indomethacin,

in doses of 5-10 mg/kg, has been reported
to significantly decrease resting (basal)
plasma renin activity in rabbits and dogs
(19,20,233). Similar doses have also been
reported to lower the level of immunoreac-
tive renal venous PGE substances, as well
as to decrease plasma renin activity in
normal and hypertensive rabbits (22,24,25,
170). Studies in normal and hypertensive
man have generally supported these obser-
vations' (171-173,234).

Indomethacin can also suppress the
rise in the levels of active renin asso-
ciated with furosemide administration
(174) and to decrease those of inactive
renin and acid-activated renin in man,
which suggests that PGs may mediate the
conversion of inactive to active renin
(175). Additionally, recent in vivo stud-
ies have confirmed these observations and
have further suggested a definite role
for newly synthesized PGs in regulating
renin release via a direct action on the

JG cells (19,20,177,178).

Recent in vivo investigations util-
lizing non-filtering-, denervated-, papa-
verine-treated canine kidneys, support the
concept of a direct action by PGs on the
JG cells to mediate renin release (28).
In these studies, 60-min infusions of
C20:4 (15 ug/kg/min) produced significant
increases in both renin release and PG
levels. On the basis of these results
it appears likely that the C20:4- evoked
increases in renin release are due to a
prior conversion of C20:4 to prosta-
glandins which then influence the JG
cells, independent of other regulatory
mechanisms.

ii. Effect of PGE_2.

In vivo and in vitro evidence sug-
gest that PGE_2 either increases or does
not alter renin release under a variety
of experimental manipulations. Utilizing
a rabbit renal cortical cell preparation,
PGE_2 at various concentrations (3×10^{-12} M,

3×10^{-10}M, 3×10^{-7}M) has been shown to increase renin release with maximal stimulation produced by the lowest concentration of PGE_2 used (9). In another study (10), a dose of 2.0 ug/ml of PGE_2 caused a gradual increase in renin release over sixty minutes of incubation, and PGE_2 doses from 3×10^{-6}M to 3×10^{-4}M have recently been shown to increase _in vitro_ renin release in a dose-dependent manner in rats (179). Furthermore, in isolated rat glomeruli, administration of 5 ug/ml of C20:4 produced a tenfold increase in PGE_2 and a twofold increase in renin release (180). In the isolated, perfused rat kidney, thirty minute infusions of 5, 10 or 50 ng/ml of PGE_2 have been reported to produce significant dose-dependent increases in renin release (181). Similarly, in the anesthetized dog, 10-min. infusions of 14-60 ng/kg/min of PGE_2 resulted in dose-dependent increases in both renin release and renal blood flow (7), while

administration of acetylcholine, bradykinin, and the depressor agent eledoisin only increased renal blood flow without affecting renin release, thus suggesting a direct action of PGE_2 on the JG cells to influence renin release (7). Yun and his associates have also reported increases in renin release in sodium deficient dogs with non-filtering kidneys, following 60-min infusions of PGE_2 (4.12 ug/min and 1.03 ug/min), further supporting the concept of a direct role for PGs in modulating renin release (15,182). Additionally, chronic intra-renal infusion of 2 ug/min of PGE_2 over 7-8 days in unilaterally-nephrectomized, conscious dogs, resulted in eight-fold increases in plasma renin activity which was attenuated following SQ 14225 administration on day 9 (183,184). Thus, taken together, these data support the view of a direct action of PGE_2 on the renin-secreting cells to influence renin release, although the mechanism involved in this action remains unclear.

In contrast, 10^{-6} M PGE_2 has been reported to be ineffective in altering renin release in rabbit renal cortical slice preparations (13,14,185). Similarly, intrarenal infusions of PGE_2 (0.1-5.0 ug/min) for sixty minutes in renal-denervated, anesthetized dogs have also failed to elicit significant increases in plasma renin activity (11), and 30 min infusions of 10-5000 ng/min of PGE_2 have also been reported to have no effect on plasma renin activity in anesthetized dogs (12). Therefore, at present the possibility of a stimulating influence of this prostaglandin on renin secretion requires further evaluation, in view of the conflicting nature of the data currently available.

iii. Effect of PGF_2-alpha.

Data pertaining to a possible influence of PGF_2-alpha on renin secretion are also conflicting. In vitro studies using rabbit renal cortical cell preparations (9), have shown that PGF_2-alpha at

various doses (3×10^{-12} M, 3×10^{-9} M and
3×10^{-7} M) significantly decreases renin
release. Furthermore, the addition of
10^{-12} M to 10^{-6} M of PGF_2-alpha to rabbit
renal cortical preparations have also re-
sulted in significant decreases in renin
release in a dose-dependent fashion (13,
14). Conversely, recent studies (10),
utilizing similar renal cortical systems
have demonstrated that 0.2 ug/ml PGF_2-
alpha stimulates renin release when the
prostaglandin was prepared in albumin-
containing physiological buffer, while
the same dose inhibits basal renin re-
lease when the vehicle was ethanol (10).
Alternatively, unilateral, intrarenal
infusions of 1.03 ug/min of PGF_2-alpha
for 1.5 hours had no demonstrable effect
on renin release in intact canine kidneys
(15).

iv. Effect of PG Endoperoxides.

Current evidence indicates that the
PG-synthetase intermediates, the

endoperoxides, are capable of stimulating renin release in vivo and in vitro. For instance, in vivo unilateral infusion of a synthetic PG-endoperoxide analogue, EPA, significantly increases renin release and this effect is prevented by prior treatment with indomethacine (186), although the rise in renin release in response to EPA administration appears to be the result of prior conversion of EPA to PGE_2 (186). In vitro studies (13,14) utilizing a rabbit renal cortical preparation have also shown that the synthetic endoperoxide analogues EPA I and EPA II and the naturally occurring endoperoxide, PGG_2, can significantly stimulate renin release to a similar extent (60% over control levels). Since in these studies PGE_2 was without effect and PGF_2-alpha actually decreased renin secretion, it appears that the PG endoperoxides themselves are responsible for the observed rise in renin secretion.

v. <u>PG-elicited Stimulation of Cyclic AMP</u>.

It is generally accepted that intra-
renal PGs stimulate renal tissue cyclic
AMP levels, but whether this is a non-
selective effect of PGs on intrarenal
adenylate cyclase systems or a specific
action on JG cell membrane adenylate cy-
clase complexes, is not clear. In iso-
lated rat glomeruli, adenylate cyclase
stimulation, has been shown to occur fol-
lowing administration of $2x10^{-5}$ M PGE_2,
while equimolar concentrations of PGF_2-
alpha were ineffective (187). Also, high-
er concentrations of PGE_2 and PGF_2-alpha
$(8x10^{-4}$ M) have resulted in a significant
stimulation of cyclic AMP content of rat
renal cortical slice tissue (188). Con-
versely, a 10-fold decrease in the concen-
tration of PGF_2-alpha resulted in the loss
of the stimulatory action of this agent on
renal cortical cyclic AMP content. Simi-
lar results have been reported in experi-
ments utilizing both renal medullary and

cortical slice tissue (189,190). Unfortunately, renin release changes were not simultaneously evaluated in any of these studies. Recently, unilateral, intrarenal infusion of dibutyryl cyclic AMP into the renal artery of anesthetized dogs, has been shown to significantly stimulate renin secretion even in the presence of indomethacin, suggesting that PG-synthetase activity does not play a role in cyclic AMP-mediated renin release (191). Indomethacin has also been reported to be ineffective in altering theophylline-induced increases in plasma renin activity in rabbits (192), while theophylline has been shown to directly stimulate PG-synthetase in rabbit renal microsomes (193). Taken together, these results suggest that the stimulatory effect of PGs on renal adenylate cyclase systems may be confined to vascular and tubular cells different from the JG cells.

vi. <u>PGs and Their Relationship to the JG Cell</u>

<u>Beta-adrenergic Receptor</u>.

Independent observations that intra-renal PGs are capable of stimulating renin release as well as renal tissue cyclic AMP content, pose the attractive possibility that the effect of PGs on renin secretion may be mediated by a membrane receptor mechanism which utilizes cyclic AMP as the intracellular second messenger, similar to the beta-adrenergic receptor mechanism which modulates the stimulatory effect of catecholamines on renin release via media-tion by cyclic AMP content changes (1-4,35, 36). This possibility is supported by <u>in</u> <u>vivo</u> studies (194-196,235), showing that indomethacin administration can suppress the anti-hypertensive effect of beta-adrenoreceptor blockade in man, cats and rabbits. However, in isolated rat glomer-uli (197), beta-adrenoreceptor blockade has failed to inhibit the increased re-lease of renin seen in response to C20:4

infusions, thus suggesting that PGs stimu-
late renin release independently of a beta-
adrenoreceptor mechanism. Furthermore, _in_
vivo studies have demonstrated the failure
of indomethacin to prevent the rise in
plasma renin activity produced by norepi-
nephrine and isoproterenol infusions in
the dog (198). Recent studies utilizing
superfused rat renal cortical slices (179),
have shown that PGE_2-induced renin release
cannot be blocked by doses of propranolol
which normally abolish isoproternol-
stimulated renin release. Thus, the bulk
of the evidence suggests that while cyclic
AMP may perhaps mediate PG-elicited renin
release, the mechanism involved in this
action may not be of the beta-adrenorecept-
or type and that the systems may function
independently of each other in regulating
renin secretion.

MATERIALS AND METHODS

Twelve male, Sprague-Dawley rats (Sprague-Dawley Co.) were fed a sodium deficient diet (Teklad Test Diet Co.) providing less than 0.02 mEq. of sodium per day for 2.5 to 3.5 weeks and had free access to distilled, deionized water. The rats were kept in a temperature- and light-controlled room (23 ± 2^0C; 12 hours light and 12 hours dark), with two animals per cage. At the end of their dietary intake period the animals were sacrified by decapitation, their kidneys placed in Robinson's buffer medium at 4^0C (199), and subsequently gassed for 30 sec. with a mixture of 95% O_2-5% CO_2 prior to and after decapsulation. Slices of renal cortex (approximately 0.3 mm thick) were prepared using a Stadie-Riggs microtome (A. Thomas Co.). Each cortical slice was further divided into several similarly-sized portions (according to the number of treatments) and then randomly assigned to separate incubation vessels containing 2.5 ml of Robinson's buffer at 4^0C. This procedure was repeated using slices from other cortical areas until each vessel contained 50 ± 30 mg of cortical tissue (wet weight). The random distribution of the sliced tissue insured that all incubation beakers contained relatively

homogenous cell populations from each cortical area. One vessel from each group was utilized as a common, non-treated control. The renal cortical tissue was then preincubated for 15 min at $37^{\circ}C$ in a shaking Dubnoff Metabolic Incubator (Precision Scientific Co.) in an atmosphere saturated with 95% O_2 - 5% CO_2. The preincubated slices were subsequently transferred to new incubation vessels containing 2.5 ml of fresh Robinson's buffer and incubated for 60 min under 95% O_2 - 5% CO_2 at $37^{\circ}C$.

Groups of beakers having a common, unstimulated control sample, were treated with either prostaglandin E_2 (PGE$_2$; 10^{-5}M) or prostaglandin F_2-alpha (PGF$_2$-alpha; 10^{-5}M) added separately or together with the beta-adrenergic receptor blocker propranolol (10^{-4}M), or the alpha-adrenergic receptor blocker phentolamine (10^{-4}M) to the renal cortical slice preparation. The prostaglandin synthetase inhibitor, indomethacin (10^{-4}M), as well as propranolol and phentolamine were also added alone to the kidney slice system.

Indomethacin, PGE$_2$ and PGF$_2$-alpha solutions were prepared in absolute ethanol to the desired concentrations. Propranolol (Sigma) was dissolves in 0.1% ascorbic acid, whereas phentolamine (CIBA) solutions were made in sterile water. The

alpha- and beta-receptor blocking agents as well as indometha-
cin were added to the tissue preparation before both the pre-
incubation and incubation periods. In turn, the prostaglan-
dins were added before the 60 min incubation period only.

Following incubation, the supernatant medium was col-
lected and stored at -20°C before being measured for renin
concentration by radioimmunoassay of angiotensin I (200).
The incubated tissue used for cyclic AMP content determina-
tion was immediately frozen on dry ice, homogenized in 8%
trichloracetic acid, and stored at -20°C, until assayed by
a modification of the competitive protein-binding assay of
Gilman (201).

Extraction and measurement of tissue cyclic AMP were
accomplished by thawing the previously frozen tissue homo-
genates in an ice-cold water bath followed by centrifugation
at 4000 rpm for 15 min at 4°C. The supernatant was then
transferred to conical centrifuge tubes while the precipitat-
ed protein was discarded. The samples were subsequently
washed four times with 2.0 ml each of water-saturated ether,
and the ether phase discarded. After the final wash, the
cyclic AMP-containing water phase was decanted into glass
vials placed on dry ice for rapid freezing. The frozen

samples were then lyophyilized and the freeze-dried residue was stored at $-20^{\circ}C$ prior to the measurement of cyclic AMP content. The values were expressed as picomoles of cyclic-AMP per mg of wet tissue.

Renin concentration in the supernatant samples was indirectly measured by determining the rate of angiotensin I generation following exposure of endogenous renin present in the collected medium, to exogenous renin substrate (angiotensinogen) obtained from 6 hr nephrectomized dog plasma, during a two-hour incubation period at $37^{\circ}C$. Angiotensinase-and converting enzyme inhibitors were added to the mixture to prevent conversion of the generated angiotensin I to angiotensin II. Briefly, the supernatant samples were allowed to thaw at $4^{\circ}C$, vortexed for 10 sec and 0.025 ml aliquots were transferred to test tubes at $4^{\circ}C$. Aliquots of 0.4 ml nephrectomized dog plasma (pH=7.56), previously treated with the angiotensinase-and converting enzyme inhibitor phenyl methyl sulfonyl fluoride (PMSF:Sigma) and 2 drops of saturated NaCl, were then added to each test tube and vortexed. The resulting mixture was incubated for 2 hr at $37^{\circ}C$, diluted 3:1 with acidified distilled water, vortexed, and then placed in a boiling water bath for 3 min to terminate the reaction. The samples

were then allowed to cool and then stored at $-20^{\circ}C$ until assayed for angiotensin I by radioimmunoassay. The results were expressed as nanograms of angiotensin I per mg of wet tissue per hr.

Statistical analysis of the data was performed by modified Student's paired t-tests (202) using a programmable desk top calculator (Hewlett-Packard) at Argonne National Laboratories (Argonne, Il.).

RESULTS

The effect of PGE_2 $(10^{-5}M)$ and PGF_2-alpha $(10^{-5}M)$ on
renin release and cyclic AMP content in renal cortical slices
from sodium deficient rats, is shown in figure 1. At the dose
used, both prostaglandins increased renin secretion by about 2
ng/mg/hr (in 5 out of 9 observations for PGE_2 and in 6 out of
10 cases for PGF_2-alpha) during a one-hr incubation period,
although the increase was not statistically significant. In
contrast, tissue cyclic AMP in response to administration of
either PGE_2 or PGF_2-alpha was markedly elevated (p 0.01) in
relation to unstimulated, control samples (significant in-
creases in 11 out of 12 instances for PGE_2 and in 10 out of
12 cases for PGF_2-alpha).

Administration of the beta-adrenoreceptor blocker, pro-
pranolol $(10^{-4}$ M), either alone or together with PGE_2 did not
alter renin release (in 6 out of 10 instances renin release
was increased by PGE_2 plus propranolol; in 11 out of 12 cases
tissue c-AMP was increased by PGE_2 plus propranolol) and did
not prevent the stimulatory effect of PGE_2 alone on the cyclic
AMP content of the renal tissue (Figure 2). Similar results

(in 5 out of 10 cases renin release was increased by PGE_2 plus phentolamine; in 11 out of 12 cases tissue CAMP content by PGE_2 plus phentolamine) were obtained when the alpha-adrenoreceptor blocker, phentolamine (10^{-4} M), was added to the renal cortical preparation either alone or in conjunction with PGE_2 (Figure 3).

Changes in renin release and tissue cyclic AMP content in response to administration of 10^{-5} M PGF_2-alpha, either alone or together with 10^{-4} M propranolol or 10^{-4} M phentolamine, are shown in figures 4 and 5, respectively. As seen previously with PGE_2, beta- or alpha-adrenergic receptor blockade was ineffective in altering renin secretion (renin release was increased in 6 out of 10 cases by PGF_2-alpha plus propranolol and in 6 out of 10 cases of PGF_2-alpha plus phentolamine) or in preventing the significant stimulatory effect of PGF_2-alpha on tissue cyclic AMP content. (Tissue c-AMP content was increased in 11 out of 12 cases by both PGF_2-alpha plus propranolol and PGF_2-alpha plus phentolamine.)

To evaluate the possibility that endogenous prostaglandins participate in the regulation of resting (basal) renin release, the prostaglandin synthetase inhibitor, indomethacin (10^{-4} M), was added alone to the kidney slice system during a one-hr incubation period (Figure 6). In the presence of this

agent, both renin release and tissue cyclic AMP content remained unaltered when compared to unstimulated control samples. (Renin release was increased in 1 out of 5 instances and c-AMP content increased in 2 out of 6 cases in response to indomethacin.)

Figure 1. Effect of 10^{-5} M prostaglandin E_2 (PGE$_2$) and 10^{-5} prostaglandin F_2-alpha (PGF$_2$-alpha) added separately, on renin release and tissue cyclic AMP content in renal cortical slices from sodium deficient rats. The data represent the mean renin release change $\pm$ SE and the mean tissue cyclic AMP content change $\pm$ SE. The number of observations (n) and the statistical significance of the observations are indicated. The mean control (nontreated) rate of renin release was 6.30 ± 1.29 ng/mg/hr and the mean control tissue cyclic AMP content was 0.34 ± 0.04 pmol/mg wet tissue. Incubation time was 60 min.

Figure 2. Changes in renin release and tissue cyclic AMP content in response to 10^{-5} M prostaglandin E_2 (PGE_2) added alone or together with 10^{-4} M propranolol (PRO) to renal cortical slices from sodium deficient rats. The data represent the mean change in renin release ± SE and the mean tissue cyclic AMP content change ± SE. The number (n) of observations and the statistical significance of the observations are indicated. The mean control (nontreated) rate of renin release for PGE_2 added alone or together with PRO was 6.30 ± 1.29 ng/mg/hr and it was 6.42 ± 1.65 ng/mg/hr for PRO added alone. Corresponding values for tissue cyclic AMP content were 0.34 ± 0.04 and 0.39 ± 0.08 pmol/mg wet tissue, respectively. Incubation time was 60 min.

Figure 3. Changes in renin release and tissue cyclic AMP content in response to 10^{-5} M prostaglandin E_2 (PGE_2) added alone or together with 10^{-4} M phentolamine (PHEN) to renal cortical slices from sodium deficient rats. The data represent the mean change in renin release $\pm$ SE and the mean tissue cyclic AMP content change $\pm$ SE. The number (n) of observations and the statistical significance of the observations are indicated. The mean control (nontreated) rate of renin release for PGE_2 added alone or together with PHEN was 6.30 $\pm$ 1.29 ng/mg/hr and it was 6.42 $\pm$ 1.65 ng/mg/hr for PHEN added alone. Corresponding values for tissue cyclic AMP content were 0.34 $\pm$ 0.04 and 0.39 $\pm$ 0.08 pmol/mg wet tissue, respectively. Incubation time was 60 min.

CHANGE IN RENIN RELEASE (ng/mg/hr) vs TREATMENT

CHANGE IN RENIN RELEASE

** Significantly Different From Control (p<0.01)

CHANGE IN c-AMP

CHANGE IN c-AMP (pmol/mg)

n=9   n=12
PGE$_2$(10^{-5}M)

n=10   n=12
PGE$_2$(10^{-5}M)
+
PHENTOLAMINE
(10^{-4}M)

n=5   n=6
PHENTOLAMINE
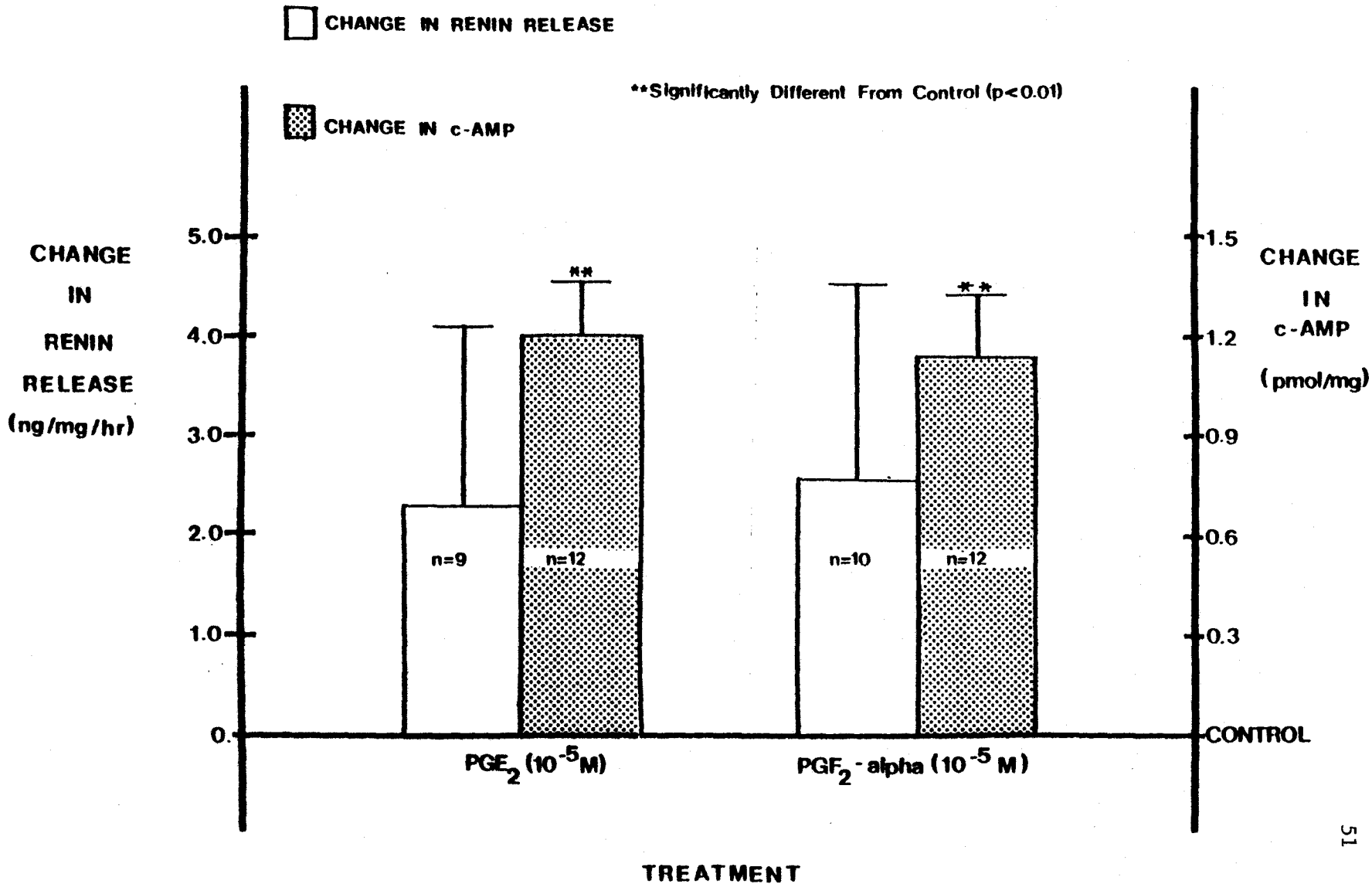(10^{-4}M)

CONTROL

TREATMENT

Figure 4. Changes in renin release and tissue cyclic AMP content in response to 10^{-5}M prostaglandin F_2-alpha (PGF_2-alpha) added alone or together with 10^{-4}M propranolol (PRO) to renal cortical slices from sodium deficient rats. The data represent the mean change in renin release ± SE and the mean tissue cyclic AMP content change ± SE. The number (n) of observations and the statistical significance of the observations are indicated. The mean control (non-treated) rate of renin release for PGF_2-alpha added alone or together with PRO was 6.30 ± 1.29 ng/mg/hr and it was 6.42 ± 1.65 ng/mg/hr for PRO added alone. Corresponding values for tissue cyclic AMP content were 0.34 ± 0.04 and 0.39 ± 0.08 pmol/mg wet tissue, respectively. Incubation time was 60 min.

Figure 5. Changes in renin release and tissue cyclic AMP content in response to 10^{-5} M prostaglandin F_2-alpha (PGF_2-alpha) added alone or together with 10^{-4} M phentolamine (PHEN) to renal cortical slices from sodium deficient rats. The data represent the mean change in renin release $\pm$ SE and the mean tissue cyclic AMP content change $\pm$ SE. The number (n) of observations and the statistical significance of the observations are indicated. The mean control (nontreated) rate of renin release for PGF_2-alpha added alone or together with PHEN was 6.30 ± 1.29 ng/mg/hr and 6.42 ± 1.65 ng/mg/hr for PHEN added alone. Corresponding values for tissue cyclic AMP content were 0.34 ± 0.04 and 0.39 ± 0.08 pmol/mg wet tissue, respectively. Incubation time was 60 min.

□ =CHANGE IN RENIN RELEASE

▨ =CHANGE IN cAMP

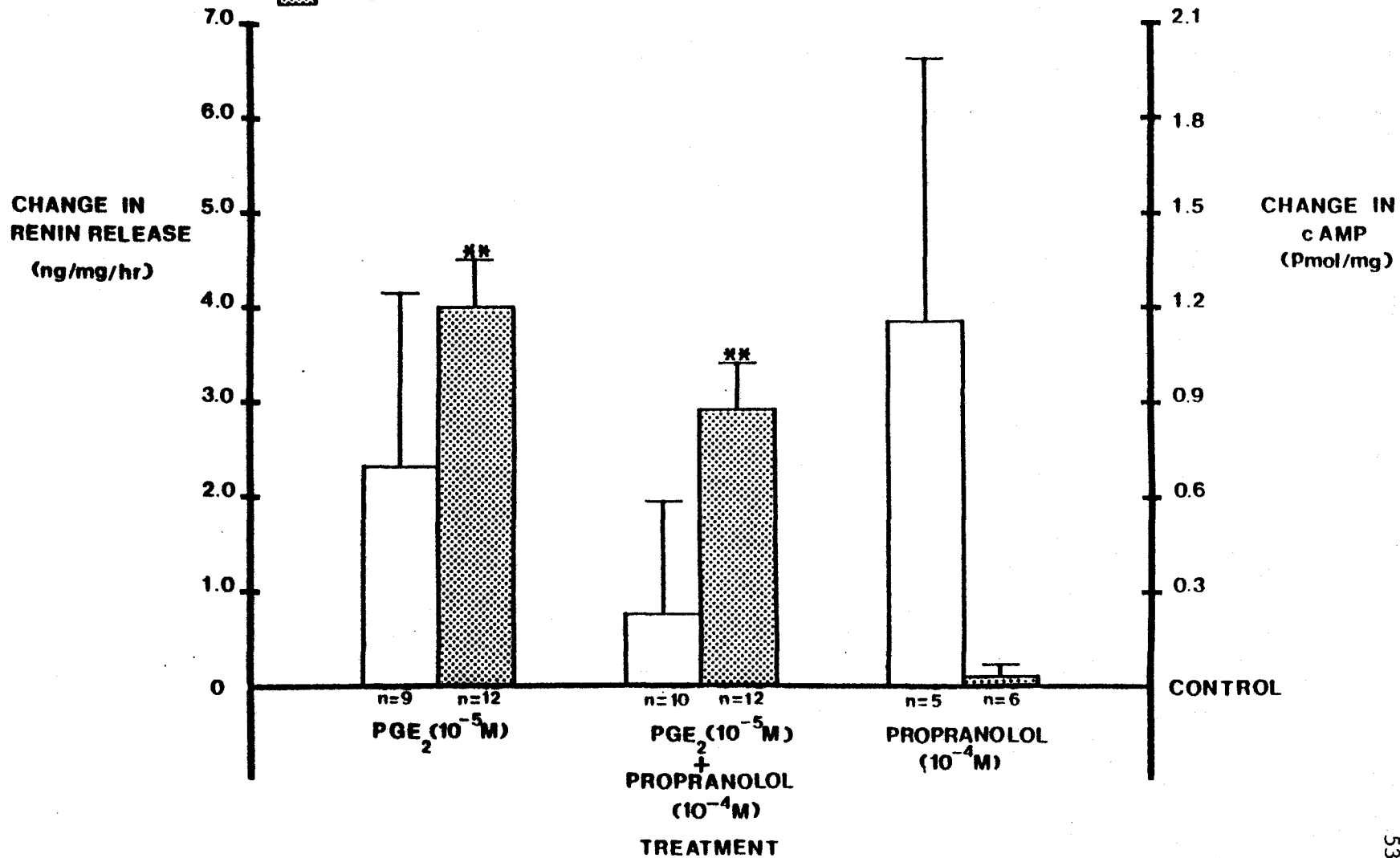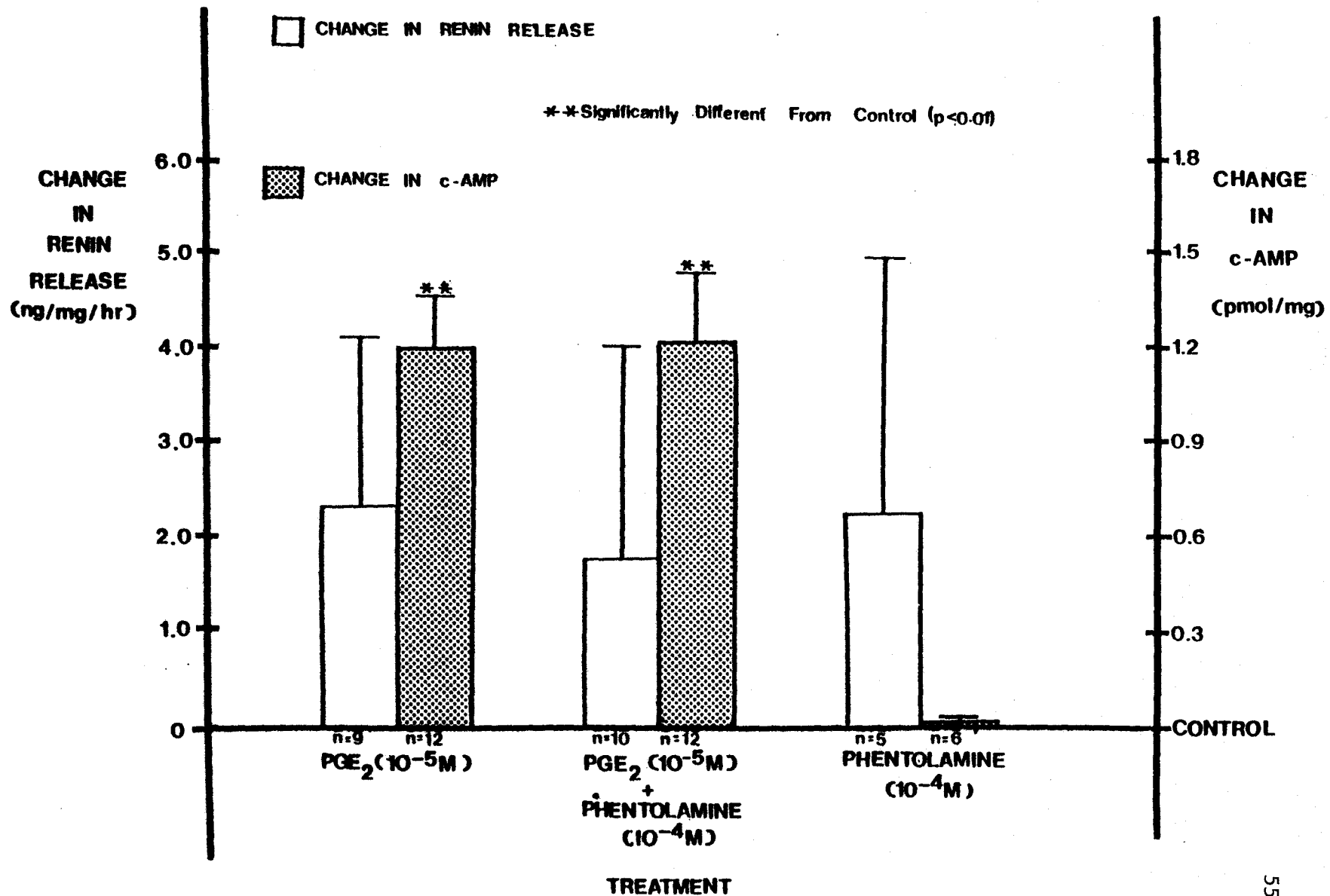** = Significantly Different From Control (p<.01)
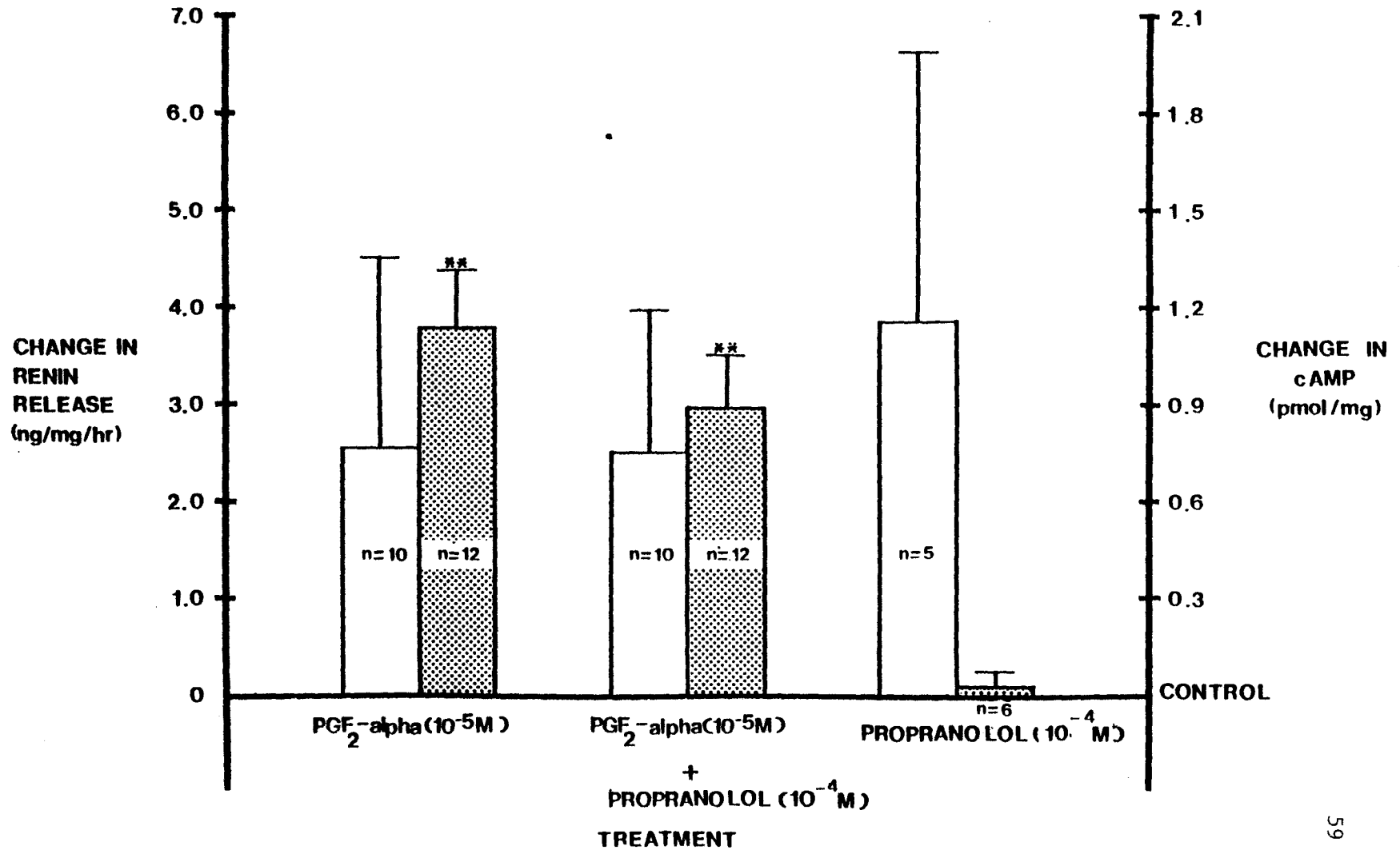
Figure 6. The effect of 10^{-4} M indomethacin (I) on resting renin release and tissue cyclic AMP content in renal cortical slices from sodium deficient rats. The data represent the mean renin release $\pm$ SE and the mean tissue cyclic AMP content $\pm$ SE of I-treated slice tissue (I) in relation to unstimulated controls (c). The number (n) of observations are indicated. Incubation time was 60 min.

INDOMETHACIN (I) 10^{-4} M

RENIN RELEASE (ng/mg/hr)

c AMP (Pmol/mg)

C ——————— I renin release

— — — — cAMP

C

I

n = 5

C

I

n = 6
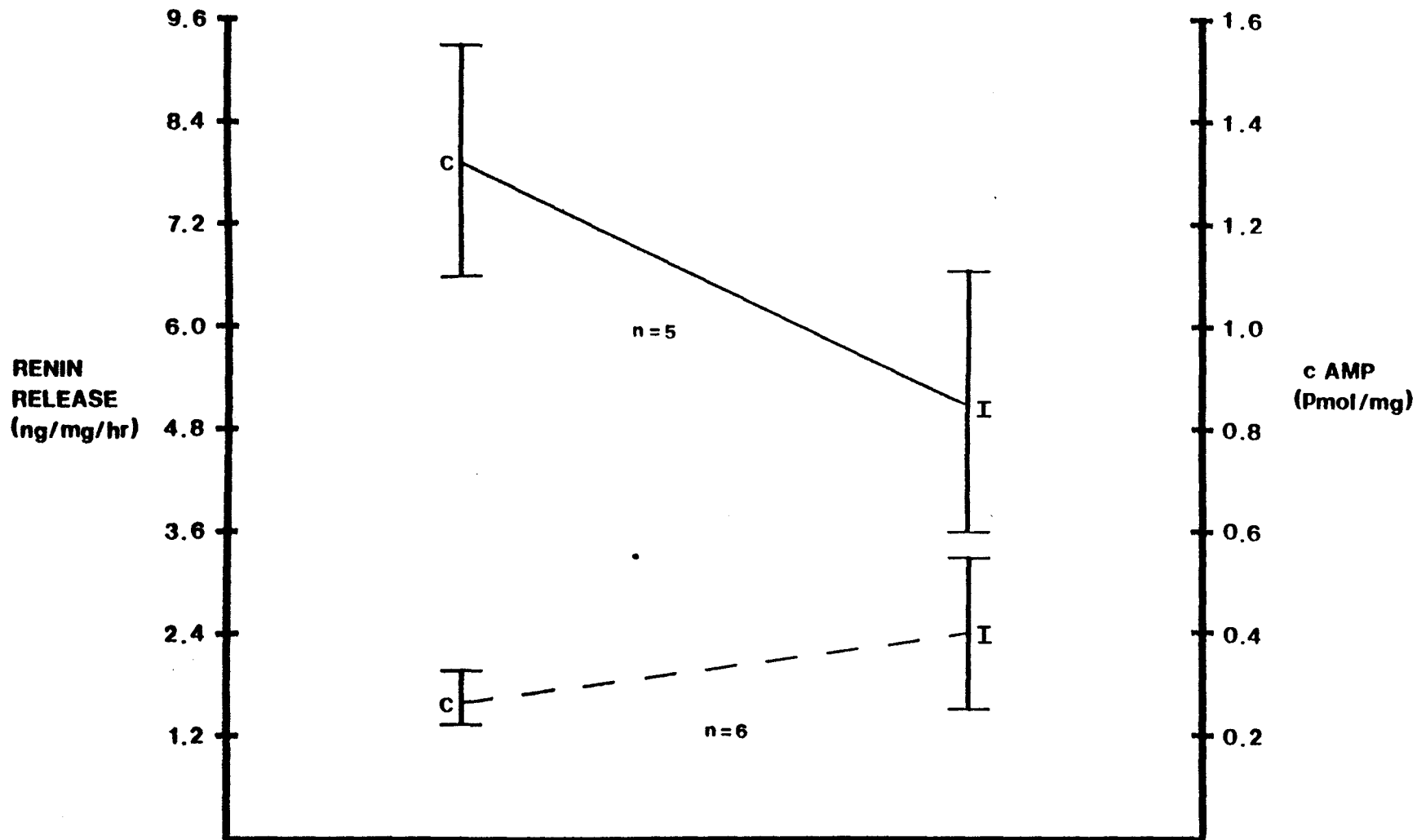
DISCUSSION

The in vitro renal cortical slice preparation has been demonstrated to be a metabolically-viable system, as judged by the linearity of renin release and tissue oxygen consumption observed with time of incubation (1,3,203-207). Utilization of this type of preparation, which is devoid of hemodynamic, humoral and other factors known to affect renin secretion in the live animal, has allowed an effective evaluation of the direct effect of a number of isolated agents on renin release (1-4,87,96,98,101,117,208-211), including those exerted by prostaglandins (9,10,13,14).

In the present study, the inability of both PGE_2 and PGF_2-alpha to significantly stimulate renin release in vitro is consistent with previous reports which suggest that administration of these agents either does not affect renin release (11,12,15) or it only exerts a mild stimulatory effect (10). Alternatively, some studies (7,179,181,182) have provided data supporting the concept that PGE_2 has the ability to significantly stimulate renin release, whereas others (9,13,14) have reported that PGF_2-alpha significantly depresses renin secretion.

One possible explanation for the conflicting nature of the data generated in these various studies, may be related to the nature of the vehicle in which the prostaglandins are routinely prepared. Whorton et al. (10) have shown that when ethanol is used as the solvent, concentrations of this agent as low as ul/5 ml of incubation medium diminish the stimulatory effect of PGE_1 on renin release by 33%, and that administration of similarly-prepared PGF_2-alpha actually inhibits renin secretion. Conversely, the same investigators have shown that PGF_2-alpha significantly stimulates renin release only when dissolved in albumin-containing physiological buffer. Although there are no corresponding data pertaining to PGE_2, the fact that absolute ethanol was utilized in the present study as the prostaglandin vehicle, may partially account for the inability of both PGE_2 and PGF_2-alpha to significantly influence renin release. A likely way by which ethanol may participate in the prevention of the stimulatory effect of these prostaglandins on renin secretion may be through its well-known dehydrating properties, which would impair the metabolic viability of the renal cortical cells. However, our observation that the cyclic AMP content of our renal cortical tissue was significantly increased, rather than decreased, by the addition

of PGE_2 and PGF_2-alpha in this study makes a detrimental effect of ethanol a less likely possibility.

To our knowledge, renin release and tissue cyclic AMP content responses to prostaglandin administration have not been simultaneously evaluated in previous studies. The consistently significant increases in tissue cyclic AMP content caused by both PGE_2 and PGF_2-alpha in this study, agree with previous observations showing a marked stimulation of renal adenylate cyclase systems by these agents (187-188). Whether or not the increase in cyclic AMP seen in those studies was coupled with corresponding changes in renin release can not be determined, since the two parameters were not measured simultaneously. Clearly, however, our data indicate that at least at the dose used in this study for both prostaglandins, their effects on renin release and tissue cyclic AMP content were independent of each other. The different effects on these two parameters caused by PGE_2 and PGF_2-alpha administration, contrast with data related to the stimulatory effect exerted by catecholamines on renin secretion. In these studies (1-4,35,36), norepinephrine and dopamine significantly stimulated both renin release and tissue cyclic AMP content in rat renal cortical tissue, suggesting that the renal receptor mechanism

involved in these stimulatory responses utilizes cyclic AMP as
a mediator. Furthermore, the same investigators have shown
that the stimulatory effect of these catecholamines on both
parameters, was effectively blocked by the beta-adrenoreceptor
antagonist, propranolol, suggesting that the type of receptor
mechanism mediating these responses is of the beta- type.
Thus, the marked stimulatory effect of PGE_2 and PGF_2-alpha on
tissue cyclic AMP content seen in the present study, may re-
flect a selective, rather than generalized, stimulation of
renal-adenylate cyclase complexes different from those located
on the membrane of the JG cells. The observation that propran-
olol administration did not alter the stimulatory effect
caused by both prostaglandins on tissue cyclic AMP content,
is consistent with this view.

A number of studies (1,35,36,115,117,212) have also
shown that renal alpha-adrenergic receptor stimulation marked-
ly depresses renin release and tissue cyclic AMP content, and
that this inhibitory effect on both parameters is effectively
converted to stimulation by administration of alpha-adreno-
receptor blocking agents. The present results indicate that
alpha-adrenergic receptors do not participate in the action
of PGE_2 or PGF_2-alpha on either renin release or tissue cyclic
AMP content, since alpha adrenoreceptor blockade by phentolamine

did not alter the changes seen in both parameters in response to administration of either prostaglandin. Thus, it appears that neither beta-adrenergic nor alpha-adrenergic receptor mechanisms, mediate the stimulatory effect of PGE_2 and PGF_2 on the renal cyclic AMP content seen in this study. Also, it is clear that at the dose used in this investigation, neither prostaglandin was capable of significantly altering resting renin release. However, before a definite conclusion regarding the possibility that prostaglandins may influence renin release can be reached, additional doses of these agents must be examine, and alternative prostaglandins such as prostacyclin (PGI_2) must be evaluated alone and in conjunction with blocking agents. Furthermore, immunohistochemical studies designed to isolate the actual site of PG-stimulated cyclic AMP content would be of great value in this regard.

In vitro studies utilizing renal cortical tissue of rabbits (13,14) have shown that indomethacin, a known prostaglandin synthetase inhibitor, significantly suppresses resting renin release, an observation which suggests that endogenous renal prostaglandins participate in the minute-to-minute regulation of renin secretion in this species. Similarly, Romero

et al. (25) have demonstrated in live dogs that indomethacin is a potent inhibitor of resting renin release. Using the same concentration of indomethacin, $(10^{-4}M)$, as these workers, we observed a mean decrease in resting renin release after 60 min of incubation, although this decrease was not statistically significant. Thus, the present in vitro data do not currently support the concept that resting renin release is under partial modulation by intrarenal prostaglandins. Alternatively, our results in the rat may be indicative of species differences or of differences in dietary sodium intake, since previous studies employed animals maintained on commercial, sodium replate diets. The inability of indomethacin to influence resting renin release in this study, was also apparent in regard to the cyclic AMP content of the renal cortical tissue. This observation further supports our view that in vitro renin secretion in the rat is not regulated to any significant extent by endogenous renal prostaglandins, since their activity should have been reflected in an increased adenylate cyclase stimulation mimicking that observed in this study upon administration of exogenous PGE_2 and PGF_2-alpha. It is clear that further studies, designed to determine whether or nor the marked stimulatory effect exerted by these prostaglandins on

renal adenylate cyclase complexes can be altered by simultaneous indomethacin administration, are required before these questions can be effectively answered.

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APPROVAL SHEET

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The final copies have been examined by the director of the
thesis and the signature which appears below verifies the
fact that any necessary changes have been incorporated and
that the thesis is now given final approval by the Committee
with reference to content and form.

The thesis is therefore accepted in partial fulfillment of
the requirements for the degree of Master of Science.

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