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ROLE OF NEWLY SYNTHESIZED STEROID HORMONE ANTAGONISTS IN ADRENOCORTICO-STEROID HORMONE-INDUCED HYPERTENSION

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A dissertation in partial fulfillment

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ROLE OF NEWLY SYNTHESIZED STEROID HORMONE ANTAGONISTS IN ADRENOCORTICOSTEROID HORMONE-INDUCED HYPERTENSION

ABSTRACT

A dissertation in partial fulfillment of the requirements for
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Excess adrenocorticosteroid hormones such as glucocorticoids and mineralocorticoids is well known to induce hypertension in several animal species as well as in humans. Therefore, the development of potent and specific glucocorticoid and mineralocorticoid antagonists with antihypertensive effects is clinically necessary. steroid hormone antagonists being used therapeutically present serious endocrinology side effects such as the widely used antimineralocorticoid, spironolactone. The antiglucocorticoids available so far have been active only *in vitro* or only weakly *in vivo*. Recently, three new exciting adrenocorticosteroid hormone antagonists have been synthesized. RU 486 is a potent antiglucocorticoid (and antiprogesterone with potential as an abortifacient), RU 26752 and mespirenone, are novel mineralocorticoid antagonists. I studied

the antihypertensive effect of RU 486, RU 26752 and mespirenone in Sprague-Dawley rats with dexamethasone- or aldosterone-induced hypertension. In addition, the effect of these antagonists on the hypertension developed by genetic model, spontaneously hypertensive rats (SHR) were also studied. The SHR is the closest animal model to human essential hypertension and it is believed that adrenocorticosteroid hormones are involved in the induction and maintenance of the hypertension. The results obtained from my studies showed that RU 486 administered simultaneously with dexamethasone prevented the hypertension induced by dexamethasone treatment. However, RU 486 had no effect on mineralocorticoid-induced hypertension. The administration of the antimineralocorticoid RU 26752 or mespirenone in combination with aldosterone successfully presented aldosterone-induced hypertension but not dexamethasone-induced hypertension. Surprisingly, RU 486 caused a significant increase in the blood pressure of the SHR whilst mespirenone caused a slight decrease in blood pressure as compared to control SHR.

The effect of these antihormones on body/organ weights, fluid intake and urinary output was observed. Morphologically examination of the heart and kidney showed no abnormalities with treatment. These results suggest that 1) RU 486 is specific in preventing dexamethasone-induced hypertension; 2) RU 26752 and mespirenone are successful in preventing aldosterone-induced hypertension and 3) mineralocorticoids may be involved in the development and maintenance of hypertension in the SHR.

INTRODUCTION

The adrenal cortex has long been known to be involved in the pathogenesis of hypertension. It has been observed that hypertension cannot be produced or sustained without the adrenal cortex as bilateral adrenalectomy without steroid hormonal replacement leads to decreased blood pressure which causes circulatory collapse and eventually death (1,14). On the other hand, it is known that adrenocortical hyper-reactivity leads to an increase in the blood pressure (2,3). Several clinical and experimental studies indicate that adrenal steroids play an important role in the development and/or maintenance of various types of hypertension (4). Steroid hormones produced by the adrenal cortex are broadly classified into two categories, namely, glucocorticoids and mineralocorticoids. The exogenous administration of high amounts of glucocorticoid agonists such as dexamethasone (5-7) and mineralocorticoid agonist such as aldosterone (8-10) induce hypertension in several animal species as well as in humans. In addition, adrenal hyperactivity is associated directly with hypertension in three disorders in humans: 1) Cushing's Syndrome, 2) Primary aldosteronism, 3) adrenocortical enzymatic defects.

Cushing's Syndrome is due to the prolonged exposure to excessive levels of cortisol, the principal glucocorticoid of the human adrenal cortex. This disease

can arise from endogenous glucocorticoid excess associated with tumors of the adrenal gland or the pituitary gland. The long-term exogenous administration of synthetic glucocorticoids used clinically to suppress inflammation and immunity in the treatment of diseases such as vasculitis, arthritis, skin diseases, malignancy, primary nephrotic syndrome, secondary glomerulonephritis and transplantation can also lead to Cushing's Syndrome (11). Since the introduction of synthetic adrenocortical steroids as therapy for these various diseases, hypertension has become the most frequent complication of long-term exposure to steroid therapy (12-14). Whatever the anatomic cause or the source, high blood pressure is the rule in these patients. However, in Cushing's Syndrome patients there is a higher incidence of hypertension with the endogenous occurring disease (80%) than the long-term exogenous synthetic glucocorticoid administration (20%) (15,16). The explanation for this observation may be due to the low or absent mineralocorticoid activity of synthetic glucocorticoids. Cushing's Syndrome is the cause of hypertension in one out of 300 hypertensives (11). This is a very aggressive disease with a 50% 5-year mortality rate if untreated (17). Even with present therapy, the mortality rate is still four times that of the general population (18). Thus, a new and more effective therapy is needed in the treatment of Cushing's Syndrome.

The second disorder, primary aldosteronism, is due to the excessive production of aldosterone independent of the renin-angiotensin system, the main regulator of aldosterone production. Primary aldosteronism is the most common form of endocrine hypertension occurring in patients, mostly women, between the

ages of 30 and 50 years. The syndrome of primary aldosteronism has been classified in four types:

1. Aldosterone producing adenoma (APA)
2. Adrenocorticol hyperplasia
   -idiopathatic hyperaldosteronism (IHA)
   -dexamethasone suppressible hyper-aldosteronism (DSH)
   -primary adrenal hyperplasia (PAH)
3. Adrenocortical carcinoma
4. Ectopic aldosterone-producing tumors

The most common type of primary aldosteronism is unilateral aldosterone producing adenoma (APA) or Conn's Syndrome which accounts for 65% of primary aldosteronism (1). APA is characterized by increased aldosterone production from a unilateral adenoma and rarely is it bilateral. There is suppression of the renin-angiotensin system (RAS) and urinary potassium wasting which results in hypokalemia. APA is the second most common cause of curable hypertension achieved through the surgical removal of the adrenal. On the other hand, the hypertension in the other three types of primary aldosteronism are not cured by adrenalectomy. The most common subtype of bilateral adrenocortical hyperplasia, idiopathic hyperaldosteronism (IHA) which accounts for 34% of the cases (19), unlike APA, the IHA occurs mostly as bilateral hyperplasia. The hypertension in 85% of these patients fails to improve with adrenalectomy which recurs within 1 to 2 weeks after surgery (19,20). Recognizing the type of primary aldosteronism is critical in the treatment of these patients as some can be cured partially or completely from hypertension by adrenalectomy while others cannot.

Therefore, other non-surgical therapeutic measures such as the use of aldosterone antagonists like spironolactone are employed.

Adrenocortical enzymatic disorder or congenital adrenal hyperplasia (CAH) syndrome is inherited in an autosomal recessive fashion and is characterized by three separate enzymatic defects in cortisol biosynthesis. The deficient enzymes in CAH, their frequency and the induction of hypertension are as follows:

Adrenocortical Enzymatic Disorders

Enzyme Deficiency	Frequency	Induction of Hypertension
21 hydroxylase	90%	No
11ß hydroxylase	8%	Yes
17α hydroxylase	1%	Yes

The 11ß hydroxylase and 17α hydroxylase deficiency lead to hypertension. The 11ß-hydroxylation deficiency is characterized by accumulation of steroids proximal to the block such as 11-deoxycorticosterone (DOC) and a reduction in 11-hydroxylated steroids such as cortisol, distal to the defect. 17-hydroxylase deficiency also increases the level of steroids proximal to the block and reduce production of cortisol distally, resulting in increased secretion of ACTH. High levels of ACTH induces the excess production of intermediate steroids including the mineralocorticoids, DOC, 18-hydroxyl DOC and 19-nor-DOC which contribute to the development of hypertension (1).

Adrenocortical steroids have been implicated in several models of experimental hypertension (21) and also in human essential hypertension,

particularly, in low renin essential hypertension (19). Essential hypertension simply means the cause(s) of the hypertension is unknown. It has been hypothesized that in essential hypertension excess production of adrenocortical steroids cause volume expansion (thus, suppression of renin production) which may contribute to the development of hypertension (22). However, several studies have shown increased (23,24) or decreased blood volume (25) in essential hypertensive subjects. Some of these patients exhibit antihypertensive response to adrenal inhibitors (26-28), mineralocorticoid antagonists (22,29) and adrenalectomy (30). The potent mineralocorticoid 19-nor-DOC has been detected in high amounts in the urine of some patients with essential hypertension (31,32). Furthermore, other studies have shown increased adrenal activity in several models of hypertension (33,35). However, direct adrenocortical involvement remains to be proven. Thus, to investigate the causes and mechanisms involved in the pathogenesis of human hypertension, different experimental models are studied. There are several models of experimental hypertension:

1. Mechanically-induced hypertension such as renovascular hypertension.
2. Genetic models of experimental hypertension.
3. Surgical manipulations such as adrenal regeneration hypertension.
4. Chemically induced hypertension such as steroid-induced hypertension which is the focus of this work.

1. Renovascular Hypertension

Goldblatt and his associates showed in 1934 that constriction of the renal artery in dogs produced and sustained hypertension (36). Therefore, this model of

experimental hypertension is also known as Goldblatt hypertension. Since then it has been shown in rats, sheep and rabbits (37,38,39). In this model, the renin-angiotensin system (RAS) has been known to play a major role in regulating the blood pressure (40,41). Renin, produced by cells in the juxtaglomerular apparatus in the kidney (due to decreased pressure in renal artery) converts angiotensinogen produced by the liver to angiotensin I. Angiotensin I is converted to angiotensin II by angiotensin converting enzyme (ACE) which is produced in the lungs. Ang II is not only a potent vasoconstricting agent but also stimulates aldosterone production from the adrenal cortex. There are two different models of Goldblatt hypertension. The first model is known as the two kidney-one clip (2KlC) which involves constricting the renal artery while the contralateral kidney is intact. The second model, is the one kidney-one clip (1K1C) in which one kidney is clipped and the contralateral kidney is removed. The mechanisms involved in increasing the blood pressure are different in these two models. The renin-angiotensin system (RAS) is believed to be responsible for the development of hypertension in the 2K1C model and not in the 1K1C model. This conclusion is based on the observation that plasma renin activity (PRA) is elevated during the pathogenesis of 2K1C hypertension (37,42,43). In addition, blocking the RAS with either AII antagonists or ACE inhibitors reverse increased blood pressure in 2K1C animals (43-45). However, sodium balance in 2K1C animals has been reported to be normal (42,46), decreased (47) or transiently increased (48,49). Unlike the 2K1C model, the 1K1C model is not dependent on the RAS. Ang II antagonists and ACE inhibitors have no effect or are less effective in reversing 1K1C *hypertension* (43,44,50,51) and studies show a positive sodium balance. On the other hand, it

has been suggested that the different mechanisms reported for the 2K1C and 1K1C models may be due to the fact that several studies fail to consider the circadian rhythm for renin and aldosterone secretion, the severity of the renal artery constriction and species differences (21).

The role of the adrenal cortex in renovascular hypertension has been investigated. It has been reported that bilateral adrenalectomy reduces renal hypertension unless there is hormone therapy (52) or saline is given (53). In contrast, adrenalectomy lowered the BP of renal hypertensive rat to normal levels even when they were maintained on saline (54). The administration of prednisolone, a glucocorticoid agonist, restored the blood pressure to hypertensive levels in adrenalectomized rats with renovascular hypertension (59). However, glucocorticoid replacement failed to increase pressure in adrenalectomized dog with renovascular hypertension (56). In adrenalectomized sheep given glucocorticoid and mineralocorticoid, 1K1C hypertension persisted for two days after withdrawal of steroid (57) suggesting that the adrenal might not be that essential in renal hypertension. Another study showed that 1K1C adrenalectomized dogs maintained on basal cortisone and 11-deoxycorticosterone (DOC) still developed hypertension (58). Since hypersecretion of these adrenocortical steroid hormones was not possible, it was concluded that the role of the adrenal was not essential for the development of 1K1C hypertension. De Jong and his colleagues (59) compared renin-induced and 2K1C hypertension in intact and adrenalectomized rat given saline. They reported that renal hypertension developed in both intact and adrenalectomized 2K1C models. Renin-induced hypertension developed in intact rats but not in adrenalectomized

rats. However, high doses of renin were able to produce hypertension in adrenalectomized rats without steroid replacement. They concluded from these results that the adrenal is not essential for renin or renal hypertension (59). Watkins and associates (58) also reached a similar conclusion using 1K1C hypertensive dogs. On the other hand, a more significant role of the adrenal gland has been suggested by Ribeiro and Krakoff (60) and other researchers (61-63). Before the infusion of saralasin, the blood pressure of 1K1C rats and adrenalectomized 1K1C was 174 mmHg and 142 mmHg, respectively, a difference of 32 mm. This difference became 68 mmHg after saralasin infusion due to a decrease in BP of the adrenalectomized 1K1C rats to 126 mmHg (but not in the intact 1K1C). Since no decrease in the BP was observed in the intact 1K1C rats with saralasin infusion, they contributed to the reduction in BP in the adrenalectomized rats to the lack of adrenals. Aldosterone level is increased throughout the development of 2K1C hypertension and only in the early stages of 1K1C hypertension (21). The involvement of glucocorticoids in the pathogenesis of renovascular hypertension is sketchy but so far studies show increased levels (64) while others observed no change (65). Thus, all these studies seem to suggest that the adrenal gland serves a prominent but secondary role in the pathogenesis of renovascular hypertension.

2. Genetic Forms of Experimental Hypertension

Experimentally, there are different types of the genetic model.

Dahl and Rapp Salt-Sensitive Rats

Dahl and co-workers selectively bred Sprague-Dawley rats based on their blood pressure response to high salt intake (66). Two strains were developed,

namely, susceptible (S) and resistant (R). The S rats developed hypertension rapidly on a high salt diet whereas the resistant rats did not. Rapp and Dahl have suggested the difference between S and R rats is due to a gene or genes controlling adrenocorticosteroids biosynthesis (67). Rapp and Dahl (68) used F_1 and F_2 generations of S x R cross to show that the relative activity of steroid 18-hydroxylase and 11-ß hydroxylase (which catalyses 18- and 19-hydroxylation of DOC) was being controlled by a single Mendelian gene with two alleles inherited in a codominant fashion. The involvement of these steroids have been investigated. It has been shown that R rats became hypertensive when connected to S rats on a high-salt diet (69). Blood taken from S rats reduced the excretion of sodium of isolated kidney from normotensive Sprague-Dawley rats (70). Adrenalectomy prevents the development of hypertension in S rats (71). The adrenal cortex in S rats show increased secretion in mineralocorticoids such as 18-hydroxy-DOC (72), 19-nor-DOC (73,74). Also, Gomez-Sanchez reported high levels of corticosterone in the urine of S rats than R rats (73). This finding contradicts the observation of Rapp and Dahl (67) that blood levels of corticosterone and the *in vitro* biosynthesis is lower in S rats than R rats. These contradictory results have been explained by the difference in the level of 11-ß hydroxysteroid dehydrogenase, the enzyme that catalyses the metabolism of corticosterone to 11-dehydrocorticosterone (75). The level of this enzyme has been found to be higher in the S rats than the R rats.

Aside from increased mineralocorticoid secretion causing hypertension in the S rats, sex and age also play a role. The development of hypertension in female S rats with high salt intake is slower (67) which is contrary to reports that

female rats have higher 18-hydroxy-DOC and 19-nor-DOC than males (73). Therefore, the degree of response to salt by the S strain depends on factors such as age, sex, duration and the amount of dietary salt intake (H).

Okamoto/Aoki Spontaneously Hypertensive Rats (SHR)

This is a strain of rats which develop hypertension spontaneously as they grow. Okamoto and Aoki reported on this strain in 1963 (76). The most interesting aspect of this strain is the possibility that SHR may be the animal model for essential hypertension in humans. Extensive studies by Aoki (77) proved how critical the adrenals are in the development of hypertension in the SHR. When young prehypertensive SHR rats (less than 8 weeks old) are adrenalectomized, thyroidectomized or hypophysectomized, the development of hypertension is prevented (77). Even bilateral adrenalectomy in SHR with established hypertension normalized the blood pressure, however, unilateral adrenalectomy failed to reduce the hypertension. This is consistent in human studies where adrenalectomy reduces blood pressure and patients with untreated Addison's diseases have hypotension (21). Aoki and colleagues observed that the adrenocortical zones were hypertrophied in the SHR (78). Thus, the adrenal gland is critical in the hypertension of the SHR.

However, other researchers do not agree totally with a critical role for the adrenal cortex in the development of hypertension in the SHR (79). Though adrenalectomy reduces the blood pressure rise, eventually hypertension was developed in the SHR due to the possible regeneration of accessory adrenocortical tissue (79,80). The role played by adrenocortical steroids have also been studied and the results are inconsistent. Several studies have shown the

involvement of glucocorticoids. Adrenalectomized prehypertensive SHR rats with glucocorticoid replacement restored hypertension while others have shown aldosterone replacement to be more important (for review see 81). Corticosterone levels at the time of significant blood pressure increase have been observed to be higher in the SHR than control rats (82), lower in the SHR (83), or unchanged (84). Recently, Hashimoto et al. (85) demonstrated the importance of the pituitary-adrenal axis indicating a more important role for glucocorticoid involvement. There has been a report of increased plasma aldosterone levels in SHR neonates (86). In addition, high levels of the potent mineralocorticoid 19-nor-DOC is found in the urine of SHR rats (87). The variability in the results of adrenocortical function in hypertension in the SHR may be due to differences in numerous factors. These factors are l) age of the SHR rat at the time of study since they develop hypertension after 8 weeks of age. Thus, it should be clear whether prehypertensive or hypertensive rats are being studied; 2) sex of the rat-normally male SHR is used in these experiments so as to eliminate the effects of other hormones during menstruation or pregnancy; 3) high salt or low salt diet; 4) stress level when blood samples were taken for steroid measurements; 5) time of day blood samples were taken as the secretion of these steroids follow a circadian pattern; 6) strain of rats used in the experiment as controls for the SHR-whether Sprague-Dawley, Wistar/Kyoto, and so on; 7) source of the SHR as there may be differences in breeding in the various laboratories.

The New Zealand Genetically Hypertensive (GH) Strain of Rat

The New Zealand GH rat was developed by Smirk and Hall in 1958 (88). These rats have significant increase in their blood pressure when they are only two

days old which progresses as they grow. Studies of adrenocortical function in these rats have focused on the renin-angiotensin-aldosterone system. However, the involvement of this system has been proved otherwise. The plasma renin activity (89) and plasma aldosterone level (90) is lower in GH rats than in control rats. In fact, inhibition of the renin-angiotensin system does not have any effect on the blood pressure of GH rats (91). Thus, the contention is that the renin-angiotensin-aldosterone system is not involved in the pathogenesis of hypertension in GH rats though other mineralocorticoids other than aldosterone could play a role. Unfortunately, there has been no reports on other mineralocorticoids or glucocorticoids effect on this model.

The Lyon Rat Strains

Three strains of rats which are either hypertensive (LH), normotensive (LN) or have low blood pressure (LL) have been developed by Vincent and co-workers (92). This was achieved by inbreeding of Sprague-Dawley rats identified to have the highest, average or lowest blood pressures. Interestingly, the hypertension that eventually develops is much less than in the SHR. Gomez-Sanchez et al. (93) have studied the role of adrenocortical steroids in the blood pressure level in the three strains. In prehypertensive rats, which is before the age of eight weeks, DOC levels increased significantly in the LH rats and decreased in LL rats compared with LH rats. Hence, DOC excretion was increased in the LH group accompanied with decreased corticosterone excretion. This observation suggests an abnormality in the adrenal cortex such as 11ß-hydroxylase deficiency.

The Milan Hypertensive Strain (MHS) of Rat

MHS comes from the Wistar strain that were selectively bred from rats with systolic blood pressures over 160 mmHg (94). Studies of adrenocortical function in MH rats show a decreased plasma renin activity in prehypertensive rats which is surprisingly followed by normal and not decreased level of aldosterone (95,96).

The Sabra Strains of Hypertension - Prone and Hypertension-Resistant Rats

These strains were selectively bred from the Hebrew University Sabro (SB) rats which had been uninephrectomized and saline-loaded with a blood pressure response to DOCA treatment. This resulted in the development of two strains. SBH that was hypertension-prone and SBN that was hypertension-resistant (97). The abnormal function of the pituitary-adrenal axis may be involved in this form of experimental hypertension as SBH rats have higher basal corticosterone levels which correlates with higher ACTH levels than in the SBN rats (64).

3. Experimental Hypertension Through Surgery - Adrenal Regeneration Hypertension (ARH)

It was demonstrated by Skelton that uninephrectomized adrenal-enucleated rats maintained on 1% saline developed hypertension (98). An intact adrenal gland or corticosterone administration prevented ARH but an intact pituitary gland was necessary for the development of hypertension (99). Plasma levels of ACTH are increased after adrenal enucleation (100,101). The regenerating adrenal gland may be secreting abnormal hormone or secreting an imbalance of normal hormone resulting in hypertension. In fact, it has been shown that regenerating adrenals secrete less corticosterone in response to stress (102) or to

ACTH (103). Not only do these glands secrete lower levels of corticosterone but also aldosterone (103-105). Further investigation into steroid synthesis of the regenerating adrenal cortex revealed a reduced ability to convert progesterone to corticosterone resulting in the accumulation of DOC which induces hypertension (106). This observation suggests an impairment in 11ß-hydroxylase enzyme in rats with ARH.

In contrast, other investigators have reported increased levels of 18-hydroxy-DOC and not DOC (107-109) whilst Rapp found a decrease in the levels of both corticosterone and 18-hydroxy-DOC (110). The inconsistency in results is due to the difference in age of rats after adrenal regeneration and in experimental protocol. High levels of the potent mineralocorticoid 19-nor DOC has been detected in the urine of rats with ARH (111).

4. Experimental Hypertension Through Chemical Intervention - Steroid-Induced Hypertension

Adrenocortical steroid hormones as discussed previously play a very important role in blood pressure control. Adrenocortical hormones are broadly classified into two major categories, namely, glucocorticoids and mineralocorticoids. Steroid hormones known as glucocorticoids tend to be ubiquitous affecting inflammation, immunity and intermediate metabolism. Mineralocorticoids are vital in electrolyte balance.

Mechanism of Action of Steroid Hormones

Steroid hormones exert their biological effects by passing through the plasma membrane and binding to specific intracellular receptors in the target tissues. When hormone binds to the receptor, the receptor gets activated and the

hormone-receptor complex gets translocated into the nucleus. In the nucleus, the occupied receptor binds to specific DNA sequences which results in the regulation of transcription of those genes in the target tissues. Steroid hormones may cause the induction or the repression of gene transcription (112). If induction of transcription occurs, mRNA produced is translated to proteins in the cytoplasm resulting in *de novo* synthesis of proteins (translation). Steroid hormones may also have non-genomic actions as some effects of these steroids are too fast with short duration to be explained by a genomic mechanism (113).

The Physiological Effects of Glucocorticoids

Cortisol is essential for life as human beings cannot survive without it. Total adrenalectomy without glucocorticoid replacement will result in death eventually (114). This hormone is ubiquitous affecting cardiovascular function, immune response, metabolism, muscle formation, behavior and almost all tissues. Basal levels of glucocorticoids, maintained by ACTH through negative feedback regulation modulate a variety of processes in the resting state. Most of the glucocorticoid effects in the resting state is termed permissive because they permit other hormones and factors to accomplish their function. Glucocorticoids increase the level of glucose by protein catabolism. It increases total body fat at the expense of protein.

They enhance water diuresis by preserving the rate of glomerular filtration. They increase vascular responsiveness and modulate central nervous system function. In addition, glucocorticoid hormones decrease lymphocytes, basophils and eosinophils while increasing neutrophils. Thus, these hormones have effect on immune response, CNS and renal function.

However, high levels of cortisol in response to stress may inhibit protein matrix formation leading to osteoporosis, and increased production of gastric acid which increases the formation of peptic ulcers. The pharmacological effects of glucocorticoids refer to their anti-inflammatory and anti-allergic actions. Glucocorticoids reduce inflammation by decreasing vascular permeability, stabilizing lysosomal membrane, inhibiting kinin production, migration and swelling of cells. The anti-allergic effects of cortisol is due to the inhibition of antibody production through lympholysis and histamine release. Therefore the excess production or administration of glucocorticoids results in a series of pathophysiological effects, the most dominant effect being hypertension. Since the introduction of the synthetic glucocorticoids in clinical use for a variety of disorders, hypertension has been observed as a major complication of long-term steroid therapy. It has been demonstrated that cortisol infusion increases blood pressure in humans (11) and hypertension is a common complication in patients with Cushing's Syndrome (2). In addition, short-term administration of ACTH to normal and hypertensive subjects raised their blood pressure, but not in patients with Addison's disease (115) indicating an important role of cortisol production in hypertension. The synthetic glucocorticoid agonist prednisone when given to postrenal transplant patients has a direct correlation between the level of arterial pressure and the dose (116). The hypertensive effects of synthetic glucocorticoids tend to be species specific. Dexamethasone administration induces hypertension in rats (5,117,118) but not in sheep (119). However, ACTH infusion quickly raised the blood pressure in sheep while cortisol, aldosterone or deoxycorticosterone (DOC) had far less hypertensive effect (11).

The Characteristics of Glucocorticoid-Induced Hypertension

Glucocorticoid-induced hypertension is not affected by a reduction in renal mass or dietary salt intake (120,121). Rats fed a high or low salt diet develop glucocorticoid hypertension (120). Dietary salt restrictions failed to abolish glucocorticoid-induced hypertension and only lowered blood pressure slightly (121). This model of experimental hypertension occurs without hypokalemia or suppression of the renin-angiotensin system. In fact, studies show that glucocorticoids increase angiotensinogen secretion (122) as well as the renin concentration (123).

Glucocorticoid treatment is associated with increased water consumption, urinary output and sodium excretion (natriuresis) resulting in a negative sodium balance (5,125). However, hematocrits of rats with glucocorticoid excess hypertension are lower, suggesting an increase in plasma volume (124). One of the hallmarks of experimental glucocorticoid-induced hypertension using dexamethasone is a great reduction in body weight caused mainly by skeletal muscle catabolism. Glucocorticoid-induced hypertension is also characterized by a rapid increase in blood pressure which is observed within a week of steroid administration (5,125).

Though excess glucocorticoids are known to increase arterial blood pressure, the biochemical mechanisms by which they induce and maintain hypertension are unclear. Several factors have been implicated in the actual pathogenic mechanisms such as enhanced response of vascular smooth muscle to pressor agents (126), activation of the renin-angiotensin system (127), fluid movement from intracellular to extracellular compartments (121) among others.

Possible Mechanisms of Glucocorticoid-Induced Hypertension

1. Electrolyte Balance

Glucocorticoid-induced hypertension does not require high dietary salt-intake (unlike mineralocorticoid-induced hypertension) (120). However, other studies using the naturally occurring glucocorticoid cortisol or corticosterone, have shown that dietary salt restriction reduced the rise in blood pressure although it did not totally prevent it (128). This observation has been explained by the fact that cortisol and corticosterone possess both glucocorticoid and mineralocorticoid actions and may raise blood pressure by both mechanisms. Free cortisol has 1% to 2% the affinity of aldosterone for mineralocorticoid receptors in the kidney (129). Thus, if cortisol exceeds the binding capacity of binding globulins and glucocorticoid receptors, the free cortisol could contribute to mineralocorticoid activity. This has been observed as a possible occurrence in some patients with Cushing's disease (1). On the other hand, synthetic glucocorticoid agonists such as dexamethasone (5,130) and RU 26988 (131) have virtually no mineralocorticoid activity and yet cause experimental hypertension. In fact, dexamethasone has antimineralocorticoid effect in the rat (132) but still raises blood pressure indicating that salt intake and thus, electrolyte balance is not essential for glucocorticoid hypertension and therefore other mechanisms may be involved.

2. Fluid Compartmentalization

Administration of glucocorticoids causes fluid shift from the intracellular to the extracellular compartments thereby increasing the extracellular fluid volume (121). Glucocorticoids activate cell membrane Na^+K^+ ATPase, an enzyme which promotes the exchange of sodium ions from the cell for potassium ions outside the

cell. The extrusion of Na^+ from the cell is followed by water increasing the volume of the extracellular compartment. The Na+/K+ APase activity is increased in erythrocytes of patients with Cushing's Syndrome (133) and those treated with ACTH (134). Glucocorticoids increase NA+/K+ ATPase in human erythrocytes (135) and in myocardial tissue of SHR and normotensive rats (136). Thus, in spite of a negative salt balance, there is a shift of water from inside to the outside of the cell resulting in hypervolemia. The immediate effect of prolonged hypervolemia will be increased cardiac output which may ultimately result in increased peripheral resistance through autoregulatory mechanisms and hence hypertension. However, it has been observed that the increase in extracellular fluid volume does not correlate well with the increase in arterial pressure (137). Since glucocorticoid-induced hypertension cannot be fully accounted for by hypervolemia, other mechanisms may be contributing.

3. Renin-Angiotensin System

Inhibition of the renin-angiotensin system (RAS) in glucocorticoid-induced hypertension reduces the high blood pressure (138,139). This indicates the role of the RAS in this model of hypertension. Glucocorticoids stimulate and maintain the action of the RAS in contrast to suppression of this system by the actions of mineralocorticoid. The formation of the renin substrate angiotensinogen is induced in the liver by glucocorticoids (140). The level of plasma angiotensinogen is raised in patients with Cushing's Syndrome (122) and in experimental models of glucocorticoid hypertension (117). Angiotensinogen is catalyzed by renin to form angiotensin I (AI) which is then converted to angiotensin II (AII), by the converting enzyme produced by the lungs. Glucocorticoids maintain this lung

converting enzyme as adrenalectomy reduces the level of the enzyme appreciably which is restored by dexamethasone but not mineralocorticoid replacement (141). In all, the angiotensin formation has been reported to be increased by about 40% due to glucocorticoid actions (123). On the other hand, others have observed that adrenalectomy had no significant effect on the *in vivo* conversion of AI to AII (142). Though in the normal subjects cortisol excess reduces plasma renin concentration due to increased plasma volume, the plasma renin activity (PRA) may be low, normal or elevated in Cushing's Syndrome patients (143,144). Administration of the AII antagonist saralasin or the converting enzyme inhibitor, captopril, decreases pressure in experimental glucocorticoid hypertension (117,138,139). When captopril is administered simultaneously with excess glucocorticoids, hypertension is delayed but not fully prevented (145). Inhibiting the RAS in patients with Cushing's Syndrome has given inconsistent results (146-148). Patients with high PRA showed markedly reduced arterial pressure, however, the pressure is still higher than normotensives (148). Therefore, the RAS may participate in glucocorticoid-induced hypertension but it does not entirely account for the elevation in arterial pressure observed.

4. Vascular Smooth Muscle Reactivity

Glucocorticoids may induce hypertension by enhancing the response of vascular smooth muscle (VSM) to pressor agents such as catecholamine and other adrenergic agonists (114). It has been shown in normal human subjects (149) and several animal species (150-152) that glucocorticoids increase vascular responsiveness to alpha-adrenergic amines. On the other hand, lack of cortisol diminishes cardiovascular sensitivity to pressor substances leading to hypotension

(114). Patients with adrenal insufficiency are hypotensive and resistant to pressor agents unless treated with adrenal steroid hormones (153). In experimental animals with bilateral adrenalectomy, VSM is insensitive to vasoconstrictors unless pretreated with glucocortiocids (154). This led to the hypothesis that cardiovascular collapse in adrenal crises may be partially due to insensitivity of VSM to vasoconstrictor agents (155).

The type of glucocorticoid agonist and dose administered are important factors in potentiating the pressor response of VSM to a particular vasoactive substance. Cortisol administration to normal subjects increases the pressor response to phenylpherine but not to AII (149). Dexamethasone given in experimental glucocorticoid hypertension enhances VSM response to NE (150) but when methylprednisolone is given, the VSM pressor response to NE or AII was not enhanced (117,139). However, selective glucocorticoid deficiency reduces pressor response to NE and AII (142). The antagonism of endogenous glucocorticoids by RU 486 result in reduced pressor response to angiotensin II (AII) and norepinephrine (NE (125).

Thus, the normal endogenous glucocorticoid concentration (within physiological range) may be required for normal vascular responsiveness but whether excess glucocorticoids corresponds to a direct increase in responsiveness to pressor agents leading to hypertension needs to be proven.

Specific glucocorticoid receptors are found in brain (156), cardiac and vascular tissues (157) of animals and the arterial smooth muscle cells in humans (158). However, glucocorticoids may have different actions in different tissues as proteins induced by glucocorticoids are tissue-specific (159). It has been

postulated that glucocorticoids may increase VSM responsiveness to epinephrine (E) by inhibiting the enzyme catechol-o-methyl transferase (COMT) thereby preventing the breakdown of E (160). The reason for this postulation stems from the observation that cortisol potentiates the pressor effect of E and not NE in experimental animals (160). Other possible explanation for glucocorticoid induced VSM responsiveness include increased Na^+K^+ pump activity and structural changes at the vascular wall (114). Although increased pressor responses are observed in VSM *in vitro* these local effects are not seen *in vivo* after glucocorticoid treatment. In humans, it has been reported that administering glucocorticoids increase pressor response to NE and E (152) whereas others observed no effect (117,161). These inconsistencies between *in vitro* and *in vivo* glucocorticoid-induced vascular response may be explained by the compensatory cardiovascular reflexes which might occur *in vivo*. Thus, the literature on VSM reactivity as a possible mechanism for glucocorticoid hypertension is inconclusive.

5. Renal Function

The role of adrenocorticosteroid hormones in the maintenance of normal renal function is well established (162). In adrenal insufficiency (Addison's diseases) both glomerular filtrate rate (GFR) and renal blood flow (RBF) are very low (11). Glucocorticoids increase GFR while total RBF remains constant thereby increasing the filtration fraction (GFR/RBF) (163). This may explain why there is an exaggerated natriuresis in patients with Cushing's Syndrome (164,165) and, also in normal individuals fed a high dietary salt intake and given cortisone (165). Unfortunately, how these changes in renal function contribute to glucocorticoid hypertension are not known.

6. Prostaglandins

The production of the vasodilator prostaglandins by white blood cells is inhibited by glucocorticoid action (150). It has been shown *in vitro* that glucocorticoids affect the biosynthesis of prostaglandins by inhibiting the release of arachidonic acid from phospholipids (166). They induce a protein, macrocortin, which inhibits phospholipase A_2, the enzyme that releases arachidonic acid from phospholipids. In arterial tissue, glucocorticoids directly inhibit the synthesis of prostacyclin (167). Prostaglandin E_2 (PGE_2) excretion from the kidneys is also reduced (150). Thus, glucocorticoids might raise blood pressure through the inhibition of phospholipase A_2 leading to a reduction in the activity of vasodilator protanoids. Dexamethasone-induced hypertension was prevented by the administration of a fish-oil diet which increases the formation of vasodilator prostaglandins (168). On the other hand, *in vivo* studies show that glucocorticoids have little effect on prostaglandin formation (169). In fact, glucocorticoids may increase the synthesis of some prostanoids by renal tissues (167,170). In addition, glucocorticoids might increase synthesis of prostaglandins through its lipolytic effect on triglycerides which increases arachidonic acid (170). Therefore, prostaglandin deficiency as mechanism of glucocorticoid-induced hypertension is not conclusive.

7. Other Cardiovascular Effects of Glucocorticoids

Glucocorticoids may also control the sympathoadrenal function by stimulating the production of the enzyme, phenylethanolamine-N-methyltransferase (PMNT) which catalyzes the conversion of NE to E. Inhibition of PMNT (171) or blockade of sympathetic ganglion (117) reduces glucocorticoid-

induced hypertension. Also, cardiac and lung beta receptors are increased by glucocorticoid treatment (172). However, there is no evidence that there is an increase in catecholamine production or sympathetic nervous system activity by glucocorticoid treatment.

Actions of Mineralocorticoids

The main action of mineralocorticoids on blood pressure is due to their effects is on salt and water balance. They increase sodium retention which raises the extracellular fluid volume and if prolonged, may increase the cardiac output leading to a high total peripheral resistance via Guyton's model of autoregulation (173). The actions of mineralocorticoids stimulate sodium transport from secretory cells such as the renal tubular cells, salivary gland, sweat glands and gastrointestinal tract. Increased sodium transport from the renal tubular cells into the extracellular fluid creates a negative intraluminal pH and subsequently potassium and hydrogen ions are excreted (3). The major mineralocorticoid is aldosterone.

Aldosterone secretion is regulated by the renin-angiotensin system and increases when extracellular volume decreases. Renin, produced by the juxtamedullary apparatus in the kidney when renal artery pressure is low, converts the substrate angiotensiongen produced by the liver to angiotensin I (AI). A converting enzyme synthesized in the lungs catalyzes the conversion of AI to AII. AII stimulates the synthesis of aldosterone from the zona glomerulosa of the adrenal cortex. The exogenous administrtion of aldosterone induces hypertension in experimental animals (9,10), as well as in humans (8). Primary aldosterone which can be due to adrenal adenoma (APA), adrenal carcinoma or bilateral

hyperplasia resulting in over production of aldosterone, is associated with hypertension (3). Another mineralocorticoid well studied in mineralocorticoid-induced hypertension is deoxycorticosterone (DOC). In congenital adrenal hyperplasia, 11ß and 17α-hydroxylase deficiency resulting in excess DOC production is also associated with hypertension (3). Furthermore, administering DOC to rats (174), pigs (179), dogs (176) and humans (177) caused a blood pressure increase. Unlike aldosterone, DOC is regulated by the ACTH levels and has been shown that in Cushing's Syndrome patients, the excessive production of ACTH results not only in hypersecretion of cortisol but also of DOC (1).

Besides aldosterone and DOC, other mineralocorticoids have been shown to induce hypertension (1). A potent mineralocorticoid, 19-nor DOC, induces hypertension in rats (178) and is suspected of playing a major role in hypertension developed by SHR (87).

Characteristics of Mineralocorticoid Hypertension

1. Unlike glucocorticoid-induced hypertension, mineralocorticoid-induced hypertension is dependent on dietary salt intake and reduced renal mass (124). Thus, a well-established model of mineralocorticoid-induced hypertension in rat is DOC administration combined with high salt intake and reduced renal mass.

2. Mineralocorticoid-induced hypertension is characterized by increased sodium retention resulting in hypernatremia followed by increased water retention.

3. Reduced hematocrit indicating increased plasma fluid.

4. Increased fluid intake (Polydipsia).

5. Increased body weight.

6. Increased potassium and hydrogen excretion resulting in hypokalemia and alkalosis, respectively.

7. Increase in blood pressure with a slow onset.

8. One of the most consistent biochemical effects of excess mineralocorticoid is suppression of the renin-angiotensin system via a negative feedback mechanism.

Whereas, mineralocorticoids increase renal sodium and fluid reabsorption resulting in increased extracellular fluid volume and gain in body weight, excess glucocorticoids is associated with a negative sodium balance and great reduction in body weight (124). However, the end result is the same, that is, increased blood pressure. Thus, the mechanisms employed by these two steroids in inducing hypertension are believed to be different although the actual pathogenic mechanisms responsible for the development and maintenance of steroid-induced hypertension are not known.

Possible Mechanisms of Mineralocorticoid Hypertension

1. Electrolyte Balance

The actions of mineralocorticoids on ion transport lead to increased sodium retention and then increased extracellular fluid volume resulting in increased cardiac output (8). The autoregulatory mechanisms set off by increased cardiac output returns the cardiac output to a normal level but at the expense of increased total peripheral resistance which leads to a rise in arterial pressure (173). Dogs treated with metyrapone (17) and pigs implanted with deoxycorticosterone acetate (18) suggest that the elevation of blood pressure is

associated with variable relative elevation in cardiac output and peripheral resistance.

2. Vascular Reactivity

Excess aldosterone causes increased sodium retention and intracellular sodium content in tissues which may lead to increased vascular reactivity and contributing further to blood pressure elevation. Aldosterone may stimulate passive and sodium pump-dependent Na^+ transmembrane movements in vascular smooth muscle (9). These effects are blocked in a dose-dependent fashion by mineralocorticoid antagonists. Several studies have shown that mineralocorticoid administration enhances vascular sensitivity to vasoactive substances such as epinephrine and angiotensin II (179) which occurs prior to increase in peripheral resistance (174). Evidence for this proposed mechanism stems from the observation that high affinity specific binding mineralocorticoid receptors are present in the cytosol of vascular smooth muscle cells (180). Though not conclusive, this suggests the possibility of a direct effect of mineralocorticoids on VSM. The most convincing evidence is from the result of Kornel et al. (181). They showed the existence in the arterial wall of an *in situ* molecular mechanism for mineralocorticoids. However, there is evidence that the action of steroid hormones on VSM is indirect (182).

3. Central Nervous System (CNS)

It has also been postulated that since high affinity, specific binding sites for aldosterone are found in various parts of the brain associated with blood pressure regulation, excess mineralocorticoids might have a direct effect on the central nervous system when they bind to these receptors (183,184).

Intracerebroventricular infusion of low doses of aldosterone induces hypertension in mononephrectomized saline drinking Sprague-Dawley rats and the simultaneous infusion of prorenone, a potent and specific mineralocorticoid antagonist, blocked the pressor effects of aldosterone (186). This study provides a strong evidence for a direct hypertensinogenic effect of aldosterone in the CNS. Other studies have shown that lesioning the anteroventral part of the hypothalamus (AV3V) prevents the development of mineralocorticoid-induced hypertension (179). It is thought that mineralocorticoids may change the intracellular sodium content of these hypothalamic cells affecting the release of agents such as vasopressin that stimulate drinking or influence vascular responsiveness (179).

Another possible mechanism of aldosterone is through the release of atrial natriuretic factor (ANF) to combat extracellular volume increase induced by mineralocorticoid actions (186). The biochemical mechanisms of ANF are not clear but it is possible some of its actions raises the blood pressure. The actions of ANF are complex but it is believed to act as an Na+K+ ATPase inhibitor counteracting the effects of aldosterone in the kidney (186). ANF stimulates an increase in the CGFR and prevents renal reabsorption of sodium and water, thus, acting as a powerful diuretic (187). In addition, ANF blunts renin release and has been shown to lower plasma renin levels in dogs (187). It also blocks the synthesis and release of aldosterone (188,189) and vasopressin (190). These effects of ANF actions promote diuresis and natriuresis and block the production and actions of the RAS and vasopressin and other pressor agents (187). Although these should lower the blood pressure, there is evidence ANF may ultimately lead to increased blood pressure (191). The natriuretic hormone not only inhibits the

sodium pump in the kidney, but in other types of cells including those of the VSM (192). Blaustein (193) has proposed that inhibition of Na+K+ ATPase activity leads to increased intracellular sodium which reduces the sodium gradient thus the $Na+/Ca^{2+}$ exchange fails to operate raising the intracellular calcium ion concentration. Calcium ions are responsible for muscle contraction and an increase will make the VSM contract more readily. Long periods of enhanced arteriolar contraction may lead to structural changes in the vascular walls with a rise in arterial pressure. In addition, changes in the ionic content of smooth muscle cells can increase contractility by enhancing their response to vasoconstrictive agents. Haddy and associates (144) observed that pump suppression increased blood vessel sensitivity to norepinephrine with elevation in blood pressure. Several researchers have observed high intracellular sodium ion concentration in various cell types from humans (193-195) and animals (196,197) with hypertension. Takeda and Miyamori has reported a significant increase in the basal levels of plasma ANF in essential hypertensive subjects as compared with age-matched normotensives (191). On the contrary, other researchers failed to demonstrate any significant difference in plasma ANF levels between hypertensive and normotensive groups (198-200). Thus, the role of ANF in the pathogenesis of hypertension is inconclusive.

In summary, the pathogenic mechanisms contributing to steroid-induced hypertension are numerous and undefined. Overall, it is likely that steroid hormones may exert their effect by binding to their receptor (receptor-mediated genomic actions) but also they can act through non-receptor mediated events termed the "hypertensinogenic" action (119). These could explain the reason why

the possible mechanisms in steroid-induced hypertension are not sufficient to account for the rise in blood pressure observed.

RATIONALE

Since excess mineralocorticoids and glucocorticoids are known to induce hypertension in several diseases, the therapeutic value of adrenocortical steroid hormone antagonists in the clinical treatment of such diseases is very important. Unfortunately, research in this area has been slow because there are no potent and specific antiglucocorticoids or anti-mineralocorticoids available. Those available are limited in their experimental and clinical usefulness as they often exhibit some agonist activity (201) or cross-reactivity with other steroid hormone receptors (201,202). Glucocorticoid antagonists may be active only *in vitro* and not *in vivo* (201) while mineralocorticoid antagonist such as spironolactone has serious endocrinological side effects due to its high affinity for the androgen and progesterone receptors (202). Thus, since the introduction of these steroid hormone antagonists, the search for new and pure glucocorticoid antagonists which are active both *in vitro* and *in vivo* and mineralocorticoid antagonists with less endocrinological side effects has been going on. Recently, researchers at Roussel-Uclaf have synthesized an exciting multifaceted steroid hormone antagonist known as RU 486[1] or mefiprestone which is the first known pure

[1]RU 486 [17-Hydroxy-11(4 dimethyl aminophenyl-1)]17(1propynyl)-estra-4-diene 3 one).

glucocorticoid antagonist which is active not only *in vitro* but also *in vivo* without any agonist effects (203). They have also synthesized a new mineralocorticoid antagonist designated RU 26752[2]. Scientists at Schering (West Germany) have recently synthesized a novel antimineralocorticoid compound known as mespirenone[3].

Since these new and potent antagonists are now available, it is of great clinical and scientific interest to study its therapeutic potential *in vivo*. Antagonists for steroid hormones are important not only for clinical use but also as tools for probing the molecular mechanisms of hormone action about which only little is known. It has previously been shown in this laboratory that RU 486 successfully prevents the hypertension induced by the long-term administration of dexamethasone (5). Thus, the long-term *in vivo* effect of RU 486 was studied in rats to show the significance of this drug. However, the specificity of RU 486 as an antiglucocorticoid in preventing adrenocorticoid-induced hypertension is not known. Thus, this study was initiated to investigate if RU 486 possesses some mineralocorticoid agonist or antagonist properties. On the other hand, no *in vivo* studies have been done with the newly synthesized mineralocorticoid antagonists, RU 26752 and mespirenone on steroid-induced hypertension until now. Though *in vitro* studies have shown increased affinity of these compounds for the mineralocorticoid receptor (MR) over the clinically used antimineralocorticoid, spironolactone, surprisingly no studies have been done to follow up the potency of

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[2]RU 2675 (7α-propyl-3-oxo-17α-pregn-4-ene 21, 17-carbolactone).

[3]Mespirenone (7α-acetylthio-15β-methylene-3-oxo-17α-pregna-1,4-diene-21,17-carbolactone).

these drugs to show if increased *in vitro* affinity for the MR is fairly correlated with their *in vivo* pharmacological action. Therefore, for the first time, *in vivo* studies have been conducted to evaluate the antimineralocorticoid effect of RU 26752 and mespirenone in rats.

The goal of this work was to investigate the antihypertensive effect of these novel antagonists since the most common complication of excess adrenocortical steroid hormone production is hypertension. The specific aims were: 1) to evaluate the specificity of RU 486 as an antiglucocorticoid by assessing its effect on mineralocorticoid-induced hypertension; 2) to study the long-term *in vivo* effect of the newly synthesized mineralocorticoid antagonists RU 26752 and mespirenone on mineralocorticoid-induced hypertension in rats to establish the significance of these compounds; 3) To assess if RU 26752 and mespirenone are specific for mineralocorticoid-induced hypertension or if they also prevent glucocorticoid-induced hypertension in rats; 4) to elucidate whether glucocorticoid and mineralocorticoid antagonists are effective in the treatment of the genetic model of hypertension; namely, the spontaneously hypertensive rat (SHR). In the literature, there are several reports implicating adrenocorticosteroid hormones in the development and maintenance of the SHR (82,85-87). The study of the SHR is also important because it is thought to be the closest model of experimental hypertension to the human essential hypertension.

MATERIALS AND METHODS

Deoxycorticosterone acetate (DOCA) d-aldosterone and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO). Aldosterone and RU 26752 custom-made pellets were obtained from Innovative Research of America (Toledo, OH), RU 38486 and RU 26752 (for injection, not pellets) were a gift from Roussel-Uclaf Pharmaceutical Company (France). Mespirenone was also donated by the Schering Chemical Company (Germany). Olive oil used as the vehicle was of the Pompeian brand. Male Sprague-Dawley rats (CD strain) weighing between 250-300g and six-week-old male spontaneously hypertensive rats (SHR) were obtained from Charles River Breeding Laboratories. The rats were housed in individual metabolic cages under environmentally controlled conditions exposed to 12 hours of light and 12 hours of darkness. Rats were given Purina rat chow and water ad libitum. Institutional Animal Care and Use Committee guidelines were followed to prevent pain during surgical procedure and throughout the experimental period.

General Methodology

Blood Pressure Measurements

Systolic blood pressure of all rats was determined by the tail-cuff method (204). An 11TC photoelectric pulse pressure transducer (MOD 29 pulse amplifier) (Woodland Hills, CA), a Grass polygraph (Model 7) (Quincy, MA), a

Narco automatic cuff inflator and rat temperature control unit (Houston, TX) were used. Animals were given at least 30 minutes to get accustomed to the constraint unit before pressure recordings were made. To get reliable data, recordings were done when animals were obviously calm as stress will increase glucocorticoid levels which can alter the blood pressure. In addition, taking into consideration the fact that steroid hormone release follows a circadian rhythm, it became imperative for recordings not to be done at the peak time (that is at 4 p.m.) and also each set of recordings should be done always at the same time of the day. Temperature around the rat tail was maintained at 30°C throughout the recording period. The systolic blood pressure (SBP) was taken. The SBP was taken only when the reading was stable. The SBP was considered stable when six consecutive clear readings were the same or were within 5 mmHg of each other. The blood pressure represent the average of at least six measurements for each rat per week. Blood pressure measurements were done once every week during the experimental period. Calibration of the drive amplifier on the Grass polygraph to set the baseline was done before each set of measurements.

All rats were weighed on the same day after blood pressure measurements had been taken. To keep stress to a minimum, rats were never weighed or injected before blood pressure recordings were done.

Injection Regimen

Olive oil was used as the vehicle. Injections were given intramuscularly or subcutaneously on the leg of the rats. Precaution was taken to avoid bleeding as bleeding would have caused leakage of the solution. Injections were not given

consecutively on the same leg so as to prevent swelling. To ensure even concentration of the injection solution, the solutions were thoroughly stirred just before each animal was injected.

Fluid Consumption and Urinary Output Recording

Fluid intake and urinary output of the rats were taken on two consecutive days each week during the experimental period. Animals were transferred to metabolic cages and had free access to Purina rat chow and water or 0.9% saline depending on the experimental model of steroid-induced hypertension. The fluid was in a graduated bottle so that fluid intake was easily determined. Graduated plastic bottles were attached to the bottom of the metabolic cages to determine urine volume. Readings were taken over a 24-hour period thus taking into account the circadian nature of steroid hormone release and their influence on fluid intake and urinary output.

An average of the values on two consecutive days represent the fluid intake and urine output of each group. Metabolic cages needed to be cleaned after each set of collection or the connection from cage to urine collecting bottle could be blocked with chow and fecal material thus underestimating the urine volume.

Samples of urine were frozen and later used in the determination of sodium and potassium concentration by flame photometry. Some values obtained were so variable even within groups that they were not useful in drawing any conclusions from them and therefore were not included in these studies.

Histological Preparation of Organs

At the end of the experimental period, the rats were anesthetized with carbon dioxide and killed by cervical dislocation. The liver, heart, kidney and thymus were quickly removed and weighed. Heart and kidney tissues were immediately placed in buffered formalin. Tissue sections were cut of the heart and kidney and stained with Masson's trichrome which is a fuchsin stain. This stain was used to identify areas of myocardial degeneration of heart tissues and interstitial nephritis of kidney tissues. The severity of myocardial degeneration and interstitial nephritis was indicated by the extent of fuchsinophilia.

Analysis of Data

All the data collected were analyzed statistically by the two-way analysis of variance (ANOVA) followed by the Scheffes' test for multiple comparisons using the STAT computer program. All graphs were done by Sigma plot.

CHAPTER 1

The Role of the Glucocorticoid Antagonist RU 486 in Adrenocorticosteroid-Induced Hypertension

Abstract

Previously, we have shown that RU 486 successfully prevented the hypertension induced by the long-term administration of dexamethasone to male Sprague-Dawley rats (5). In the present study, we determined the effect of RU 486 on two other experimental models of hypertension in the rat, namely, the deoxycorticosterone acetate (DOCA)-salt and the spontaneously hypertensive rats (SHR). Uninephrectomized saline-drinking male Sprague-Dawley rats were divided into 3 groups and given either 0.2 ml olive oil (control), 1 mg DOCA, or 1 mg DOCA+ 10 mg RU 486 dissolved in 0.2 ml olive oil every third day for a period of three weeks. Within a week of steroid administration, there was a sharp increase in the systolic blood pressure (SBP) in the DOCA-salt (170 ± 2.8) and DOCA + RU 486 (173 ± 1.6) treated rats over the control rats (127 ± 3.5) which remained elevated throughout the experimental period. There was significant difference in the water consumption and urinary output between the control and DOCA + RU 486 treated rats. In the experiment involving the SHR, the rats were divided into groups I, II, and III and given 0.2 ml olive oil (control), 1 mg RU 486, and 5 mg RU 486 dissolved in 0.2 ml olive oil respectively, for 3 weeks.

Instead of a decrease in the blood pressure as might be expected, surprisingly RU 486 enhanced it significantly within three weeks of drug administration. Control and 5 mg RU 486 treated rats had SBP of 153 ± 1.7, and 172 ± 2.5, respectively, during the three-week experimental period. Water intake, urinary output, body and organ weights were comparable in all groups. It is concluded that RU 486 has no effect on DOCA-salt model of hypertension but interestingly, elevates the hypertension in the SHR.

Introduction

Until recently, antiglucocorticoids available were active only *in vitro* or weakly active *in vivo* (201). A new glucocorticoid antagonist denoted RU 486 is the first known potent antiglucocorticoid active *in vitro* as well as *in vivo* in different target tissues (203). RU 486 given orally (205), intraperitoneally (206) and intramuscularly (207) results in antiglucocosticoid responses. Early studies of RU 486 treatment in patients with Cushing's Syndrome showed a partial or complete reversal of hypokalemic alkalosis found in these patients which is due to the mineralocorticoid activity of excess cortisol produced in these patients (208). This has led to the speculation that RU 486 and/or some of its metabolites might have some antimineralocorticoid activity. This speculation is valid because steroid hormone antagonist often cross-react with other hormone receptors. RU 486 not only binds the glucocorticoid receptor but has a strong affinity for the progesterone receptor and to a lesser extent to the androgen receptor (209). But *in vitro* studies have shown negligible cross-binding of RU 486 with the rat renal mineralocorticoid receptor (209). Since progesterone possesses antimineralocorticoid activity (210), it is possible that RU 486 may have some mineralocorticoid agonist or antagonist activities dependent on its antiprogestin properties. In addition, RU 486 may exert its apparent antimineralocorticoid effects via its antagonism of glucocorticoid action. Naturally occurring glucocorticoids may potentiate the mineralocorticoid sodium retention effect by increasing the extracellular volume and the glomerular filtration rate (GFR) (121,163). A glucocorticoid antagonist such as RU 486 might then show

antimineralocorticoid activity by decreasing the ECF and GFR and thus, amount of water and sodium delivered to the distal and collecting tubules. Therefore, RU 486 may be exhibiting anti-mineralocorticoid activity indirectly through its antagonism of the actions of these other hormones. Studies show that depending on the response examined, RU 486 can act both as a suboptimal or optimal antagonist. Steroid hormone antagonists may possess partial agonist activity and such antagonists are referred to as "suboptimal inducer" or a partial agonist-antagonist" and their action is known as "suboptimal antagonism." An optimal antagonist is therefore devoid of agonist activity and it is able to antagonize completely the effects of an optimal agonist. Some studies have reported that RU 486 exhibits no agonist activity even when administered in large doses (see 211 for review) while others have observed mild glucocorticoid agonist activity (212).

Therefore, RU 486 may have some mineralocorticoid agonist or antagonist properties dependent or independent of its antiglucocorticoid and antiprogestin properties. Although *in vitro* studies have shown negligible cross-binding of RU 486 with the rat renal mineralocorticoid receptors (209), this may not correlate well with its *in vivo* potency as the compound may be modified by its pharmacokinetic and metabolic activities that may produce reactive metabolites which determines its overall biological activity.

Previously, we have shown in this laboratory that RU 486 was able to prevent the hypertension induced by long-term administration of dexamethasone to Sprague-Dawley rats (5). However, the specificity of RU 486 in preventing adrenocorticoid-induced hypertension is not known. For example, could RU 486

have any agonistic or antagonistic mineralocorticoid effect in the rat? Therefore, the specificity of RU 486 in preventing glucocorticoid-induced hypertension was investigated by studying its effect on two other models of experimental hypertension, namely, the DOCA-salt and the spontaneously hypertensive rats (SHR).

Materials and Methods

Blood pressure measurements and the weights of the rats were taken on days 0, 7, 14 and 21. For initiating DOCA-salt-induced hypertension, the Sprague-Dawley rats were right uninephrectomized under ether anesthesia. A week after uninephrectomy, the animals were divided into three groups of eight rats each (n=8) and the baseline blood pressure measurements were taken. Steroid administration was by intramuscular injections every third day for 18 days (total 7 injections). Group I (n=8) received 0.2 ml olive oil/300 g, Group II (n=8) received 1 mg DOCA/300 g and Group III (n=8) received 1 mg DOCA + 10 mg RU 486/300 g dissolved in 0.2 ml olive oil. The SHR animals were also divided into 3 groups and blood pressure taken on days 0, 7, 14 and 21. Injections were given intramuscularly every third day for 18 days as above. Group I (n=8) was given 0.2 ml olive oil/150 g, Group II (n=8) received 1 mg RU 486/150 g and Group III (n=8) received 5 mg RU 486/150 g dissolved in 0.2 ml olive oil.

Fluid consumption and urinary output were recorded on two consecutive days each week (total of six recordings). At the end of the experimental period, animals were anesthetized with carbon dioxide and sacrificed by cervical dislocation. The heart, kidney, thymus and liver were quickly removed and

weighed. The heart and kidney tissues were histologically prepared for morphological examination. All data collected were analyzed by the two-way analysis of variance followed by the Scheffe's test for multiple comparisons.

Results

Results presented in Figure 1 demonstrate the effect of RU 486 on dexamethasone-induced hypertension (Am. J. Physiol. 256:E683) which serves as an introduction to this work. Results presented in Figure 2 and Table 1 show that within a week of steroid administration, there was a sharp increase in the blood pressure in the DOCA-salt treated rats (170 ± 2.8) over the control rats (127 ± 3.5) which remained elevated over the three weeks of experiment. The simultaneous administration of DOCA + RU 486 (173 ± 3.1) was unable to prevent or potentiate the hypertension induced by DOCA-salt treatment.

No significant difference in the weights among the groups was noted during this three-week period (Table II). Data shown in Table II demonstrate a significant increase in saline consumption in the rats injected with DOCA + RU 486 (66 ± 3.5) as compared to control rats (41.50 ± 2.0). This was followed by a correspondingly higher urine output in the former rats (23 ± 3.2). There was no significant difference observed in the weight of kidney and heart among the three groups.

When male SHR were given RU 486 (comparable to those given to DOCA + RU 486 on weight basis) for 3 weeks by injection (Fig. 3 and Table III) instead of decreasing the blood pressure, RU 486 significantly increased it (172 ± 2.5) over the controls (125 ± 2.1). However, RU 486 had no significant effect on body

weight, water intake, urinary output and organ weight of the SHR animals (Table IV).

Morphological examination of histologically prepared sections of the heart and kidney showed no abnormalities, that is, fuchsinophila of the myocardium and renal tissue were considered to be insignificant (data not shown).

Discussion

Ru 486 is known to bind strongly to the glucocorticoid receptor with an affinity three times higher than the potent glucocorticoid agonist dexamethasone (205,209). But it also cross-reacts with the progesterone receptor and slightly with the androgen receptor (209). Progesterone prevents glucocorticoid-induced hypertension and potentiates natriuresis through its antimineralocorticoid activity (210). Since progesterone possesses both antimineralocorticoid and antiglucocorticoid properties, this led to the notion that RU 486 may possibly have some antagonist or agonist mineralocorticoid activities as well (5). The results presented here clearly indicate that RU 486 does not possess mineralocorticoid antagonist or agonist properties. The lack of mineralocorticoid antagonist property of RU 486 in terms of its prevention of DOCA-salt induced hypertension is consistent with other *in vitro* studies suggesting that RU 486 has no cross-reactivity with mineralocorticoid receptors (209). According to Teutsch and Costerousse the 17α-propynyl side chain provides RU 486 with pure glucocorticoid activity devoid of mineralocorticoid activity and thus, this side chain may explain the lack of binding of RU 486 to the mineralocorticoid receptor (213). In addition, Grunfeld and Eloy (1985) have observed that sodium and potassium

balance was not affected by RU 486 treatment in rats which indicates it has no mineralocorticoid activity (125). On the contrary, RU 486 administration normalized the blood pressure and reversed the hypokalemic alkalosis seen in Cushing's Syndrome patients (212). Since glucocorticoid- induced hypokalemic effect is thought to be due to the mineralocorticoid activity of endogenous glucocorticoids binding to Type I receptors in the kidney, it led to the speculation that RU 486 and/or some of its metabolites might possess some antimineralocorticoid activity in man (212). Though studies have not been done in human kidneys, *in vitro* studies have shown that RU 486 has negligible cross reactivity with the rat kidney mineralocorticoid receptors (209). Due to species variability, it is necessary for similar study to be done in humans before any conclusive remarks can be drawn. On the other hand, several researchers have shown that the potent glucocorticoid agonist dexamethasone, with negligible mineralocorticoid activity cross-react with the type I receptors in the brain (184,214). However, *in vivo* physiological mineralocorticoid target tissues such as the kidney, intestine and the parotid are highly aldosterone-selective, in contrast with the hippocampus and heart (215). Thus, the binding of glucocorticoids to type I receptors tends to be tissue specific. *In vitro* studies have shown high levels of 11ß-OH steroid dehydrogenase activity in these aldosterone-selective tissues, but this enzyme has not been found in the hippocampus (216). This enzyme converts glucocorticoids to its 11-dehydro metabolite which has a low affinity for the type I receptor (217). This has been postulated as the mechanism by which

glucocorticoids are excluded from binding to type I receptors in mineralocorticoid target tissues (215).

Since RU 486 may not bind to kidney mineralocorticoid receptors, it is more likely that the apparent antimineralocorticoid activity of RU 486 is not by its binding to mineralocorticoid receptors in the rat kidney but rather indirectly through its antagonism of glucocorticoid activity. Therefore, an antiglucocorticoid preventing increased GFR will decrease the rate by which water and sodium pass through the distal and collecting renal tubules and thus decrease water and sodium retention giving the illusion of antimineralocorticoid activity. Results from this laboratory (5) and others (212,218) have shown that RU 486 prevents the transient diuresis and natriuresis induced by glucocorticoid treatment. RU 486 had no mineralocorticoid agonist property, since RU 486 did not potentiate the blood pressure in the DOCA + RU 486 treated rats in spite of a possible increase in plasma corticosterone level due to increased ACTH levels. Glucocorticoids exert a negative feedback effect on ACTH secretion from the anterior pituitary which serves as the major control mechanism of the pituitary-adrenocortical system. Studies in humans and other primates have shown that RU 486 was able to antagonize the suppressive effect of dexamethasone upon the pituitary-adrenal axis (219,220). Thus, ACTH secretion is increased during RU 486 administration. RU 486 treatment has been observed to activate the hypothalamic-pituitary adrenal axis in rats and monkeys, as well as in humans (see 203 for review). Chronic treatment with RU 486 in rats and monkeys resulted in increased ACTH levels, adrenal weights and glucocorticoid levels (203). In humans, RU 486

administration increased the ACTH secretion only at the times of the day when endogenous corticotrophin-releasing hormone (CRH) was high, subsequently, glucocorticoid levels were elevated in the early morning hours in Cushing's Syndrome patients treated with RU 486 (208). Thus, though the corticosterone level might have been increased in the DOCA + RU 486-treated rats, the elevation in SBP between these rats and the rats treated with DOCA alone was the same. This can be explained by the rationale that there were no receptors available for corticosterone to bind as the type I receptor was occupied by excess administration of DOCA and the type II by excess administration of RU 486.

DOCA given in combination with RU 486 resulted in a significant increase in saline consumption followed by increased urine output. Mineralocorticoid-induced hypertension is characterized by increased saline intake. Structures in the forebrain such as the anteroventral third ventricle (AV3V region) which is involved in the regulation of thirst, sodium homeostasis, blood volume and the secretion of arginine vasopressin (AVP) is necessry for mineralocorticoid hypertension but not in the SHR (179,221). Lesioning of the AV3V region prevents the development of this experimental model of hypertension in rats (222). It is being hypothesized that mineralocorticoids may alter the intracellular sodium content of the cells in the AV3V region and affect the release of other agents that stimulate drinking. The plasma level of vasopressin is elevated in DOCA-salt hypertension (222). The fluid consumption of rats treated with a combination of DOCA and RU 486 was even higher than rats treated with DOCA alone. *In vitro* studies have shown that RU 486 down regulates type 1 receptors in

the mouse hippocampus (184). Since angiotensin stimulates fluid intake, RU 486 seems to behave as a weak agonist centrally (185) and the secretion of vasopressin, we are postulating that RU 486 might be acting as an agonist in the rat brain, thus potentiating the fluid consumption in these rats. This increase in water consumption resulted in increased urinary output without any significant change in the weight of the animals.

Though antiglucocorticoid RU 486 did not prevent the hypertension induced by DOCA, surprisingly it enhanced the blood pressure of the SHR. Thus, our finding contradicts the conclusion of Hashimoto et al. (85) that corticosterone is essential for the development of hypertension in SHR. If this were to be the case, RU 486 treated SHR should have shown a reduced rather than enhanced blood pressure. It is important to point out that highly variable results have been obtained regarding plasma levels of adrenocorticoids in SHR by various investigators. Some have reported increased (82) or normal corticosterone (84) levels and other have reported increased (86) or decreased aldosterone (226) levels. Keeping this in mind, our results are consistent with the observation that adrenalectomy of young prehypertensive rats prevented the increase in blood pressure and subsequent infusion of aldosterone induced hypertension in these rats (81), indicating that mineralocorticoids seem to be more important in the induction of hypertension in the SHR model. To explain the enhanced blood pressure observed in RU 486-treated SHR, we are postulating that in SHR, RU 486 was bound to type II receptors blocking glucocorticoid action while type I receptors were unoccupied so that the excess corticosterone which might have

been produced by RU 486 treatment (through activation of the hypothalamic-pituitary adrenal axis) could cross-bind to type I to exert biological effects including increased blood pressure. This explanation agrees with Funder et al. (215) suggesting that in peripheral mineralocorticoid target tissues such as the kidney, aldosterone and corticosterone might have similar (patho) physiological effect mediated by type I receptors provided that glucocorticods can gain access to type I receptors. There seems to be an operational difference between Type I receptors in the central nervous system and the periphery. Whereas the aldosterone and corticosterone appear to have similar biological effect on blood pressure when they bind peripheral Type I receptors, their effects mediated by central Type I receptors are opposite (185).

In conclusion, RU 486 has no effect on DOCA-salt model of experimental hypertension but significantly increased the hypertension in SHR suggesting that 1) RU 486 specifically inhibits glucocorticoid-induced hypertension and 2) it is likely that mineralocorticoids are involved in the development and maintenance of hypertension in the SHR.

Figure 1: Effect of glucocorticoid agonist dexamethasone and the antagonist RU 486 treatment on systolic blood pressure (SBP). Each point represents mean ± SEM of six male Sprague-Dawley rats. *$p<0.05$ compared with control rats.

FIGURE 1

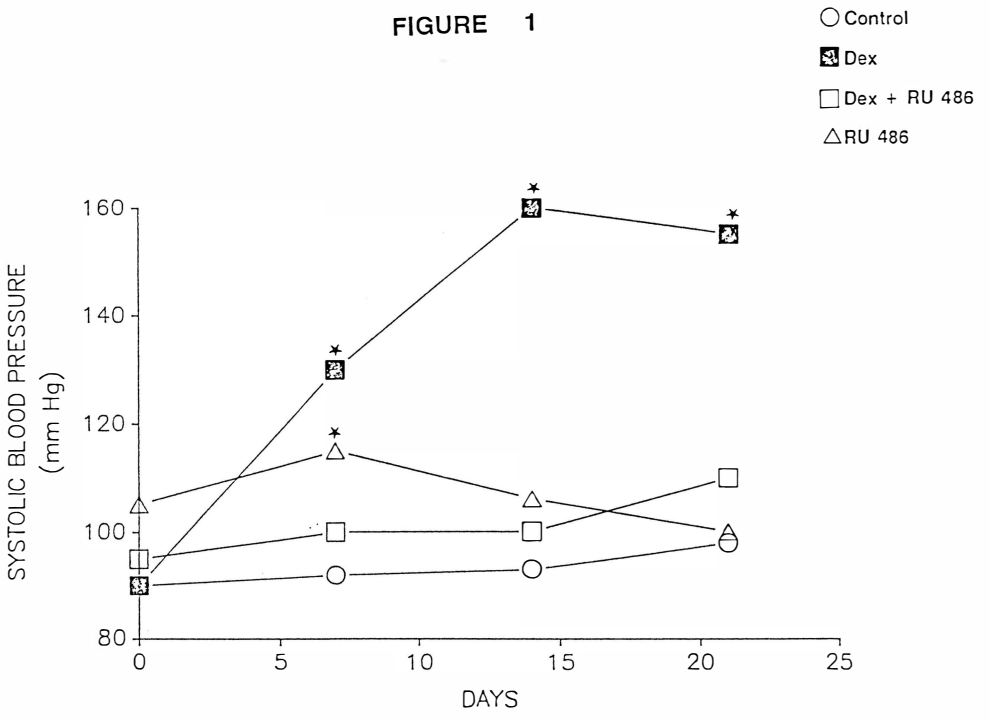
Control
Dex
Dex + RU 486
RU 486
SYSTOLIC BLOOD PRESSURE (mm Hg)
160
140
120
100
80
0
5
10
15
20
25
DAYS

Figure 2: Effect of mineralocorticoid agonist DOCA, and glucocorticoid antagonist RU 486 treatment on SBP. Each point represents mean ± SEM of eight male Sprague-Dawley rats.

FIGURE 2

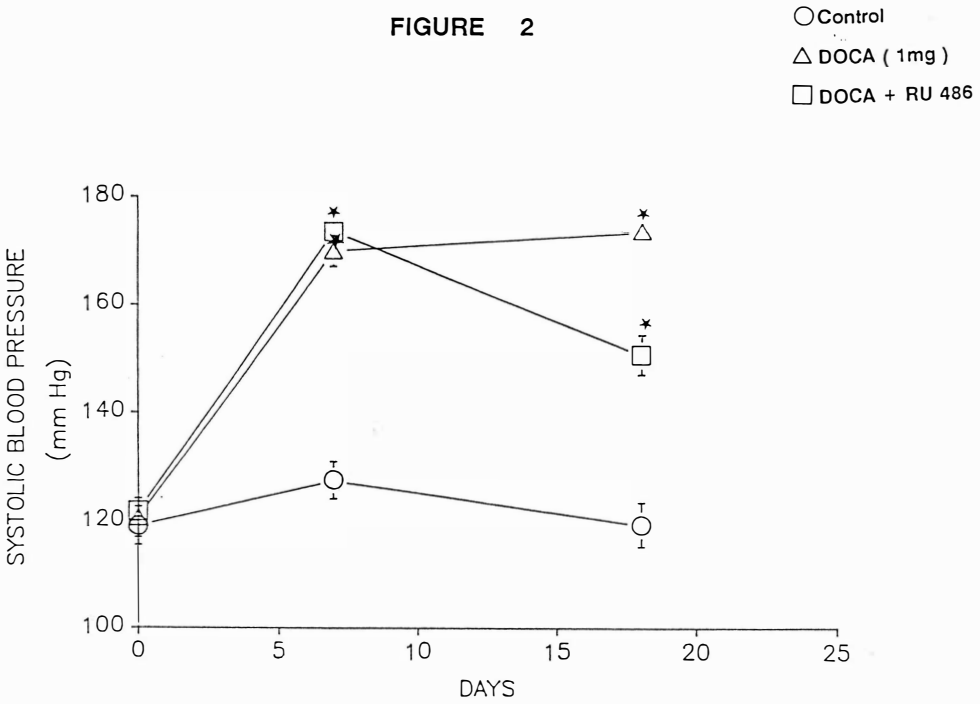
Control
DOCA (1mg)
DOCA + RU 486
SYSTOLIC BLOOD PRESSURE
(mm Hg)
180
160
140
120
100
0
5
10
15
20
25
DAYS

Figure 3: Effect of gluococorticoid antagonist RU 486 treatment on SBP of spontaneously hypertensive rats (SHR). Each point represents mean ± SEM (n=8). *$p<0.05$ compared with control rats.

FIGURE 3

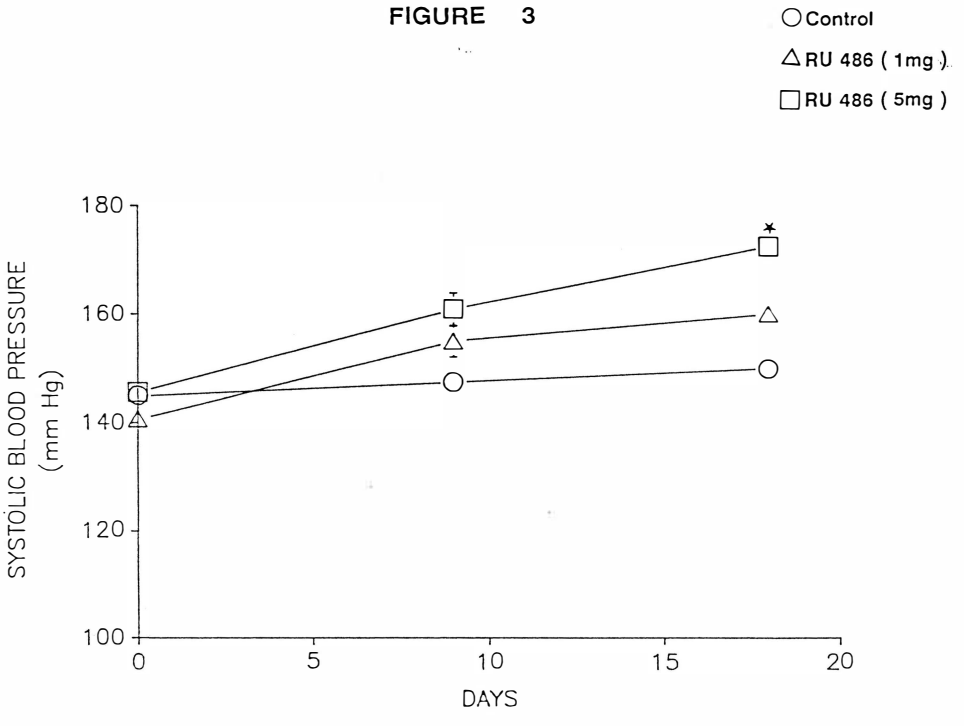
Control
RU 486 (1mg)
RU 486 (5mg)
SYSTOLIC BLOOD PRESSURE
(mm Hg)
180
160
140
120
100
0
5
10
15
20
DAYS
*

TABLE I

Effect of DOCA and DOCA plus RU486 on body weight, water intake, urine output and organ weight on Sprague-Dawley rats.

	Control	DOCA	DOCA + RU486
Body Weight (gms)			
day 0	305 ± 3·6	307·9 ± 4·4	292 ± 5·4
day 8	280 ± 9·0	281 ± 11·9	278 ± 5·6
day 20	309 ± 9·7	295 ± 11·9	308 ± 9·1
Saline intake (ml/day)	21·8 ± 2·0	54·25 ± 2·8	66·25 ± 3·5*
Urine volume (ml/24h)	14·5 ± 1·9	15·5 ± 2·27	23 ± 3·2*
Kidney weight (gms)	1·52 ± ·082	1·98 ± 0·079	1·99 ± ·09
Heart weight (gms)	0·95 ± ·037	1·005 ± 0·032	1·044 ± ·034

TABLE II

Effect of RU486 treatment on weight, fluid intake, urine volume and organ weights on spontaneously hypertensive rats (SHR).

	Control	RU486 1mg	RU486 5mg
Body Weight (gms)			
7 days	189 ± 4·9	193 ± 3·8	190·5 ± 3·5
14 days	213 ± 3·9	232 ± 4·2	213 ± 4·5
22 days	232 ± 4·1	240 ± 5·7	235 ± 3·7
Water intake (ml/day)	21·80 ± 0·87	21·30 ± 1·09	23·48 ± 1·3
Urine volume (ml/24h)	5·07 ± 0·42	5·17 ± 0·398	4·5 ± 0·364
Liver weight (gms)	8·8 ± 0·44	8·76 ± 0·43	9·2 ± 0·18
Kidney weight (gms)	1·56 ± ·039	1·78 ± ·013	1·71 ± ·024
Heart weight (gms)	0·97 ± ·035	0·87 ± ·032	0·928 ± ·034
Thymus weight (gms)	0·24 ± ·014	0·17 ± ·008	0·22 ± ·0075

CHAPTER 2

The Role of the Mineralocorticoid Antagonist, RU 26752 in Adrenocorticosteroid-Induced Hypertension

Abstract

The effect of mineralocorticoid antagonist RU 26752 on the development and maintenance of hypertension produced by long-term administration of mineralocorticoid agonist aldosterone has been investigated. Uninephrectomized, saline-drinking male Sprague-Dawley rats were subcutaneously implanted with either placebo (control) pellets, 100 μg aldosterone pellets, 50 mg RU 26752 pellets or 100 μg aldosterone + 50 mg RU 26752 pellets. Aldosterone treatment resulted in an increase in blood pressure to 165 ± 5 mmHg over the control value of 105 ± 2 mmHg within 3 weeks of experimental period. RU 26752 given alone had no observable hypertensinogenic effect. However, RU 26752 administered with aldosterone significantly prevented the hypertension produced by aldosterone alone. RU 26752 when given with aldosterone was able to prevent the aldosterone-induced increase in saline consumption, increase urine output and reduce urinary Na+ excretion. Microscopic examination did not show any significant lesions in either heart or kidney of the four groups examined. RU 26752 (7.5 mg or 15 mg) administered to Sprague-Dawley rats was unable to

prevent hypertension produced by the administration of dexamethasone. The results presented suggest that long-term administration of the antimineralocorticoid RU 26752 *in vivo* to Sprague-Dawley rats prevents the aldosterone-induced hypertension and not dexamethasone-induced hypertension.

Introduction

Presently, two steroidal aldosterone antagonists are being used clinically in the treatment of mineralocorticoid excess diseases. The widely used antimineralocorticoid is spironolactone. Spironolactone was the first competitive aldosterone antagonist used as an orally active drug.

in vitro studies show that spironolactone in ten times higher concentration is needed to displace ^{3}H-aldosterone from the receptor binding sites (202). This suggests that the relative affinity of spironolactone *in vitro* compared to aldosterone. Studies demonstrate that spironolactone also inhibits aldosterone synthesis stimulated by ACTH, potassium or Ang II (223). Although the effect on aldosterone synthesis is evident *in vitro*, the decrease in plasma and urinary aldosterone levels during spironolactone treatment is only transient (223). Thus, the natriuretic effect of spironolactone *in vivo* appears to be mainly due to its antagonism of the mineralocorticoid receptors in the target tissues. However, spironolactone is not specific for the mineralocorticoid receptor. It has affinity for the androgen and progesterone receptors as well (202). This has resulted in numerous endocrinological side effects with spironolactone treatment. Spironolactone inhibits menstruation in rabbits, monkeys (224) as well as in women (202) indicating a progestational activity of the drug. It also interferes with the secretion and the peripheral action of androgens. Administration of large doses of spironolactone causes a significant reduction in the synthesis of testesterone (202). This effect may be due to spironolactone inhibiting the 17-hydroxylase and 17-20-desmolase activities. This antiandrogenic effect of spironolactone might explain the impotence and gynecomastia observed in men

treated with spironolactone (224). Thus, the therapeutic value particularly during long-term treatment with spironolactone is limited.

The second aldosterone antagonist used clinically is potassium canrenoate which has been shown to possess a lower antiandrogenic activity in men (225). However, the antimineralocorticoid potency of potassium canrenoate is lower than that of spironolactone (202). In addition, the progestogenic potential of canrenone, the active metabolite of potassium canrenoate, is higher than spironolactone (227).

Therefore, both spironolactone and potassium canrenoate are far from being the ideal aldosterone antagonists and the search for a more specific mineralocorticoid antagonist with reduced adverse side effects has been necessary.

Several chemical modifications have been made on the spironolactone molecule in search of a better mineralocorticoid antagonist with a markedly reduced affinity for the androgen and progesterone receptors while the affinity for the mineralocorticoid receptor is increased.

The affinity of 24 spironolactone analogues to the rat renal cytoplasmic aldosterone receptor *in vitro* has been studied by Funder et al. (228). They demonstrated a reduction in affinity for aldosterone receptor in analogues with 1) unsaturation at C_6/C_7 position in ring B; 2) unsaturation of the α-lactone; 3) opening of the α-lactone ring. Substitution of the 7-α-thioacetyl group of spironolactone by a propyl residue has yielded a compound designated RU 26752. This compound is synthesized by scientists at Roussel-Uclaf. RU 26752 has been shown to possess increased specificity for the mineralocorticoid receptor over spironolactone (229). Interestingly, *in vitro* studies from this laboratory

demonstrated that the kinetics of ^{3}H-RU 26752-mineralocorticoid receptor binding, suggested two classes of receptor sites in the rat kidney (230). One class is of high affinity, low capacity in the 1-10 nM range followed by another class with a low affinity high capacity range in the 10-100 nM ^{3}H-RU 26752. The latter class of receptors have little affinity for aldosterone. The increased specificity of RU 26752 was confirmed by the observations that there was no binding to other serum carriers from organs that are not a target for the mineralocorticoid hormones and also by the lack of displacement of ^{3}H-RU 26752 by cold steroids (230). However, the correlation between the relative affinity of RU 26752 and its *in vivo* pharmacological activity is not known. Therefore, this study was conducted to confirm the *in vitro* studies demonstrating increased specificity of RU 26752 to the mineralocorticoid receptor. For the first time, the effect of long-term *in vivo* administration of this novel synthetic compound on the development and maintenance of aldosterone-induced hypertension in rats was studied. Further studies were conducted to show if RU 26752 prevents only mineralocorticoid-induced hypertension without exhibiting any glucocorticoid agonist or antagonist properties by its effect on dexamethasone-induced hypertension.

Materials and Methods

For initiating aldosterone-induced hypertension, animals were right mononephrectomized under ether anesthesia and maintained on Purina Chow and 1% saline *ad libitum.* A week after mononephrectomy, a small incision under ether anesthesia was made and custom-made pellets containing either placebo (n=6), 100 μg aldosterone (n=6), 50 mg RU 26752 (n=6) or 100 μg aldosterone plus 50 mg RU 26752 (n=6) were inserted subcutaneously. Custom pellets were

used to deliver the constant dose of steroid agonist, antagonist and combination of agonist and antagonist. The pellets were obtained from Innovative Research of America (Toledo, OH). These pellets conveniently released the desired drug *in vivo* within three weeks in a controlled dose-dependent manner.

For initiating dexamethasone-induced hypertension, four groups of rats (n=8) were given IM injections of either 0.2 ml olive oil/300 gm, 1.5 mg dexamethasone/300 gm, 1.5 gm dexamethasone + 7.5 mg RU 26752/300 gm or 1.5 mg dexamethasone + 15 mg RU 26752/300 gm, dissolved in 0.2 ml olive oil every third day for 18 days (total 7 injections).

Systolic blood pressure (SBP) was measured on days 0, 7, 14 and 21. The body weight, water intake and urine output were monitored. The Na+ concentration in the urine was measured by flame photometry. At the end of the experimental period, the animals were exposed to carbon dioxide and immediately killed by cervical dislocation. The organs were removed quickly and weighed. The kidney and heart tissues were preserved in formalin and histological preparation was done later for morphological examination. Analysis of variance was carried out and means were analyzed by Scheffe contrasts for comparison.

Experiments conducted with the SHR were not successful and due to drug and animal constraint, the experiment could not be repeated.

Results

First, we determined the optimum dose of aldosterone required to produce a significant increase in blood pressure in mononephrectomized, saline drinking rats. It was observed that 100 μg aldosterone pellets gave a significant increase in blood pressure (162 ± 4 mmHg) over control animals (107 ± 2 mmHg) within a

three-week period (data not shown). Therefore, 100 μg aldosterone pellets were used in subsequent experiments. Next, we determined the optimum dose of RU 26752 needed to antagonize the hypertension produced by the 100 μg aldosterone pellet (which delivered about 5 μg aldosterone/day). Mononephrectomized saline drinking rats were subcutaneously inserted with 100 μg aldosterone pellet and then daily injected with either 500 μg, 1 mg or 2.5 mg RU 26752 in 0.2 ml olive oil for three weeks. We noted a significant decrease in the blood pressure in the aldosterone-treated animals injected with 2.5 mg of RU 26752 along with 100 μg aldosterone. Results obtained with 50 mg of RU 26752 pellet (which releases 2.5 mg RU 26752/day) are therefore presented in Figure 4. This figure shows the differences among control, aldosterone, RU 26752 and aldosterone plus RU 26752-treated rats over three weeks. While the blood pressure of mononephrectomized, saline-drinking control and RU 26752-treated rats was slightly increased, blood pressure was significantly elevated in the aldosterone-treated (165 ± 5 mmHg) as compared to control rats (105 ± 3 mmHg). This increase was significantly prevented by simultaneous administration of antimineralocorticoid RU 26752 with aldosterone (112 ± 4 mmHg).

Data presented in Table III show that there was no significant difference in weight gain among the four groups. Saline intake and urinary excretion of water were higher during 11 and 18 days in aldosterone-treated rats as compared to control rats. On the other hand, RU 26752 and RU 26752 plus aldosterone-treated animals maintained water intake and urinary output at the level of control animals.

Urinary sodium excretion was found to be significantly reduced in the aldosterone-treated animals on day 11 but returned to control level on day 18 (Table III). No significant changes in urinary Na+ excretion were noted in RU 26752 or RU 26752 plus aldosterone-treated animals as compared to control animals. Microscopic examination of the hearts and kidneys from the four experimental groups showed no significant abnormalities.

Results presented in Figure 5 demonstrates that dexamethasone treatment to Sprague-Dawley rats resulted in a rapid increase in the blood pressure. By day 18, dexamethasone-treated animals had attained a blood pressure of 158 ± 4 compared to 118 ± 4 observed for the control (untreated) animals. RU 26752 at either 5 times or 10 times the amount of dexamethasone (1.5 mg) was unable to reverse the hypertension produced by dexamethasone during the three-week experimental period (Fig. 5). Dexamethasone-treated animals lost significant weight during three weeks of steroid administration and RU 26752 has no significant effect on reversing the observed weight loss induced by dexamethasone (Fig. 6).

Dexamethasone treatment resulted in increased water consumption, urinary output, and a decrease in liver, kidney, heart and thymus wet weight over control untreated animals (Table IV). RU 26752 at two different doses given in combination with dexamethasone was unable to reverse any of the above effects induced by dexamethasone treatment.

Discussion

Although DOCA-salt is a well-established model of experimental mineralocorticoid hypertension in the rats, we have chosen aldosterone

administration combined with high salt intake and reduced renal mass as a model for mineralocorticoid-induced hypertension in the present study. We have chosen aldosterone-induced hypertension because the majority of clinical mineralocorticoid-dependent hypertension is due to an excess of aldosterone and not DOC (3). Though hypersecretion of DOC occurs in congenital adrenal hyperplasia (CAH), such cases are very rare (9). Moreover, very large doses of DOC which is well above physiolgoical levels is required to induce mineralocorticoid hypertension in experimental animals. Since this is the first time the antimineralocorticoid RU 26752 was being used to antagonize the *in vivo* actions of mineralocorticoids we thought it necessry to use a more potent mineralocorticoid, aldosterone which is needed in microgram quantities to induce hypertension as compared to milligram quantities of DOCA (174-176). Other researchers have used aldosterone infusion to show a dose-dependent change in blood pressure of rats (9). Some studies show that the hypertensinogenic potency of aldosterone is superior to DOC in the rat as hypertension is produced by the administration of aldosterone doses which results in plasma levels no greater than that caused by stress (231).

In this study, we present evidence suggesting that aldosterone-induced hypertension in uninephrectomized salt-drinking Sprague-Dawley rats can be prevented by the simutaneous administration of the antimineralocorticoid RU 26752. While an effective dose ratio of 1 μg aldosterone: 26.8 mg spironolactone has been reported in rats (232), we have shown in this study a ratio of l μg aldosterone; 0.5 mg RU 26752 to be effective in preventing aldosterone-induced hypertension in rats. RU 26752 unlike the antialdosterone potassium prorenoate

which had a higher affinity for the mineralocorticoid receptor than spironolactone *in vitro* but was shown by Funder et al. to have a very low affinity for the receptor *in vivo* (228). This confirms the *in vitro* studies demonstrating that RU 26752 binds the mineralocorticoid receptor with greater affinity and specificity than that of spironolactone (229).

However, a rather high concentration of RU 26752 is still neeeded to antagonize the hypertensive effect of aldosterone *in vivo*. The ratio of aldosterone to RU 26752 is 1:500. In this regard, it is interesting to note that Lazar and Agarawal (230) reported a distinct anti-mineralocorticoid binding site in rat kidney which was physicochemically different from the well-characterized mineralocorticoid receptor in various target tissues. They showed evidence for a mineralocorticoid receptor with low affinity, high capacity that binds antagonist but not agonist. Therefore, it is likely that *in vivo*, a larger amount of RU 26752 is needed to exert its effect on high affinity aldosterone binding sites. Alternatively, it is possible that RU 26752 may be converted *in vivo* to a metabolite(s) with reduced biological activity therefore larger amounts are required.

Aldosterone when given alone significantly increased saline consumption and urinary output with low Na^+ excretion compared to control animals. Increased saline intake and subsequent polyuria characteristic of mineralocorticoid hypertension have been shown in several studies (179,221,222). In all these studies the AV3V region of the brain which regulates thirst, sodium balance and blood pressure has been implicated. Destruction of the AV3V region has been shown to reverse hypertension caused by mineralocorticoids and high salt intake

(179,221). However, Gomez-Sanchez has suggested that the prevention of DOCA-salt hypertension by the lesioning of AV3V region may be due to destruction of specific pressor functions and not so much as the destruction of thirst and salt appetite centers (233). On the other hand, many studies have demonstrated that mineralocorticoid hypertension does not develop if sodium intake is restricted (120,121,124) and therefore the salt appetite center is important in this model of experimental hypertension. RU 26752 administered together with aldosterone reversed the increase in saline consumption and Na^+ retention induced by aldosterone. RU 26752 was devoid of any pressure effect when given alone and had no effect on either saline consumption, diuresis or on Na^+ excretion. Thus, RU 26752 seems to have no mineralocorticoid agonist properties *in vivo*. Also RU 26752 was observed to have no deleterious effects when given alone or together with aldosterone on either kidney or heart as noted under microscopic examination. Morphological examination of the heart and kidney tissues is relevant in assessing the toxic side effects of these steroid hormone antagonists as antihypertensive drugs. It is a well-established clinical fact that a sustained and signficant reduction in arterial pressure is followed by the development of heart failure or myocardial infarction in hypertensive patients (234). In addition, experimental myocardial infarction in SHR was associated with prolonged reduction in blood pressure (235). The side effect from most antihypertensive drugs is renal failure and the histology is that of acute interstitial nephritis (236).

It is important to know whether RU 26752 is specific for mineralocorticoid-induced hypertension or it also has glucocorticoid antagonist properties. Our

results suggest that RU 26752 administered at 7.5 mg (5x the dose of dex) or even at 15 mg (10x dose of dex comparable to dose of RU 486 by ratios) is devoid of any glucocorticoid agonist or antagonist properties as it had no effect on glucocorticoid-induced hypertension. Thus RU 26752 seems to possess pure antimineralocorticoid properties in terms of its hypertensive effects in steroid-induced hypertension.

Figure 4: Effect of mineralocorticoid agonist aldosterone and antagonist RU 26752 treatment on SBP. Each point represents the mean ± SEM of two separate experiments using Sprague-Dawley rats (n=6) in each experiment. *$p<0.05$ compared with control rats.

FIGURE 4

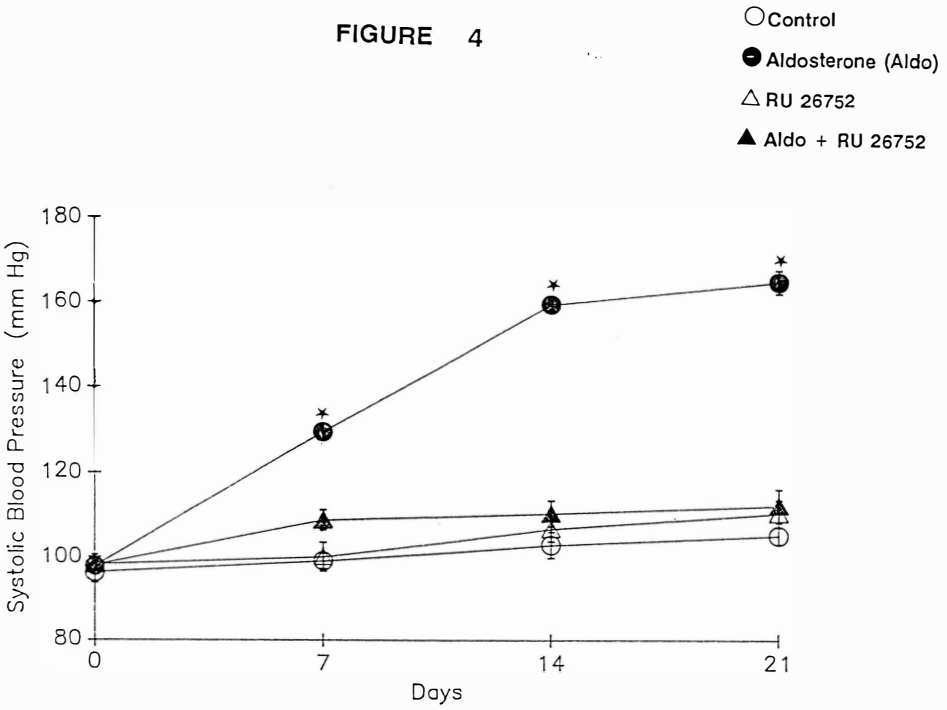
Control
Aldosterone (Aldo)
RU 26752
Aldo + RU 26752
Systolic Blood Pressure (mm Hg)
180
160
140
120
100
80
0
7
14
21
Days

Figure 5: Effect of mineralocorticoid antagonist RU 26752 on dexamethasone-induced hypertension. Each point represents the mean ± SEM using Sprague-Dawley rats (n=8) in each group. *$p<0.05$ compared with control rats.

FIGURE 5

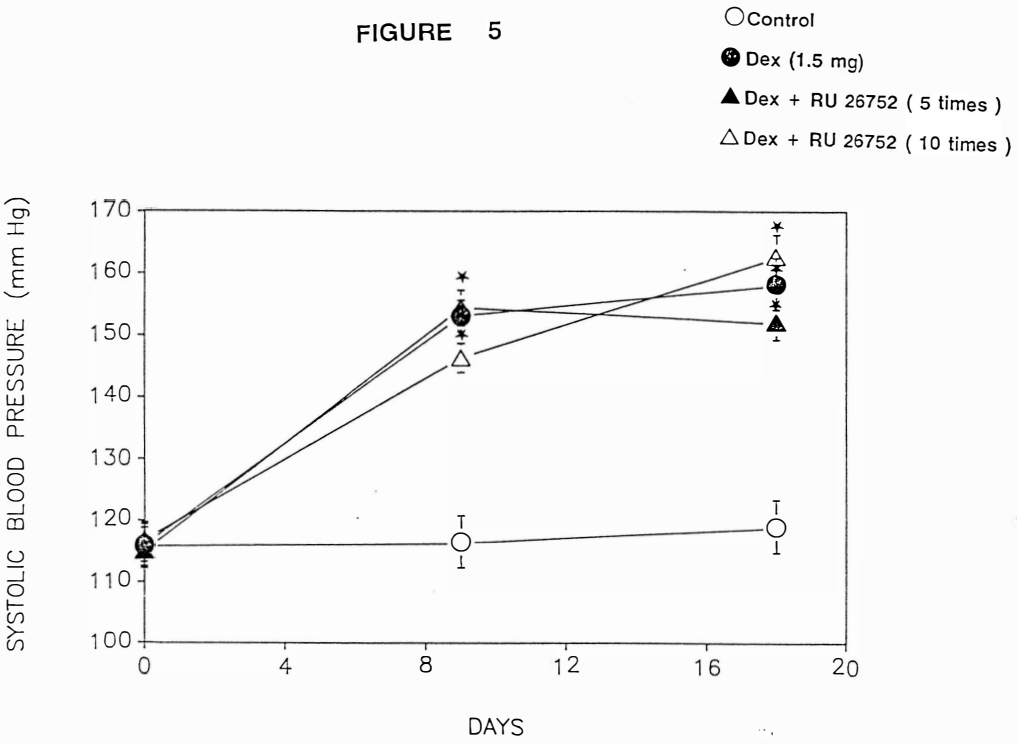
Control
Dex (1.5 mg)
Dex + RU 26752 (5 times)
Dex + RU 26752 (10 times)
SYSTOLIC BLOOD PRESSURE (mm Hg)
170
160
150
140
130
120
110
100
0
4
8
12
16
20
DAYS

Figure 6: Body weight in dexamethasone and dexamethasone plus RU 26752 treated rats. Each point represents the mean ± SEM using Sprague-Dawley rats in each group. *Significant differences from corresponding value in control untreated animals ($P<.05$).

FIGURE 6

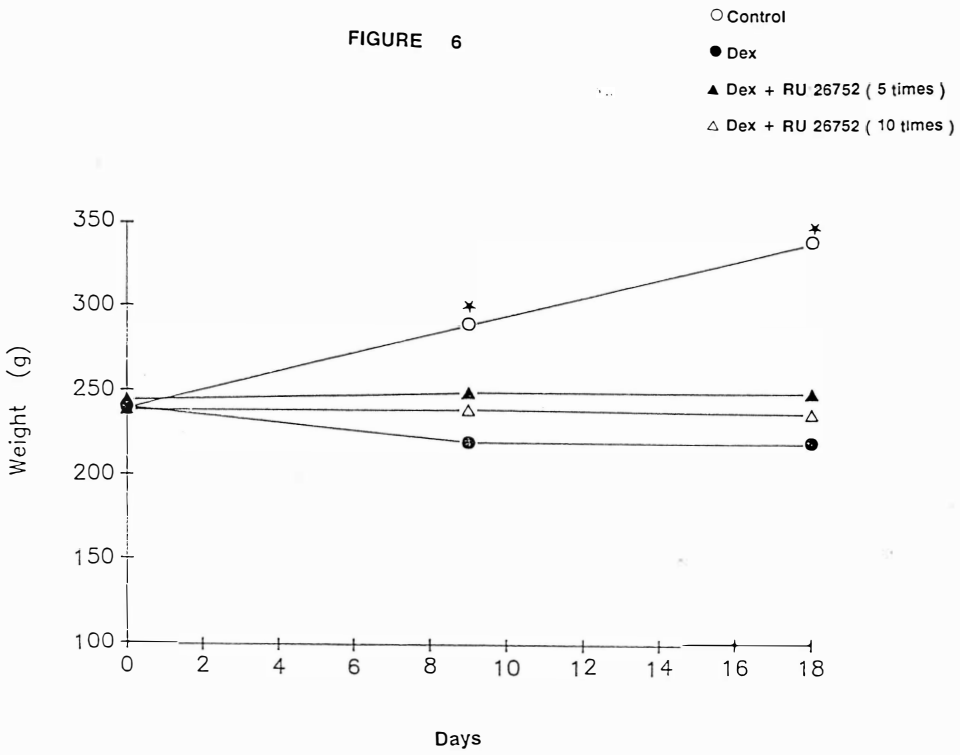
○ Control
● Dex
▲ Dex + RU 26752 (5 times)
△ Dex + RU 26752 (10 times)
Weight (g)
350
300
250
200
150
100
0
2
4
6
8
10
12
14
16
18
Days

Table III

Effect of mineralocorticoid agonist aldosterone and antagonist RU 26752 treatment on body weight, saline intake, urine output and excretion of sodium.

		Days after treatment	
Body Weight (g)	0	11	18
Control	225±6	248±7	282±4
Aldosterone	234±8	252±6	304±4
RU/26572	228±8	261±7	280±5
RU/26752 + Aldosterone	242±10	274±9	306±7
Saline Intake (ml/24h)			
Control	28±2.1	30.2±2.6	38.6±3.2
Aldosterone	29±2.6	*52.2±1.8	*51.2±2.8
RU/26752	31.2±3.1	35.2±1.6	36.1±3.1
RU/26752 + Aldosterone	26.4±2.4	38.8±2.1	39.2±1.8
Urine output (ml/24h)			
Control	16.8±1.6	14.3±1.2	17.1±1.5
Aldosterone	17.6±2.1	*24.8±3.2	*31.2±4.1
RU/26752	15.2±1.4	18.2±1.6	17.6±1.8
RU/26752 + Aldosterone	17.4±2.1	19.3±1.4	19.8±1.3
Sodium (meq/24h)			
Control	2.4±0.3	2.8±0.2	2.4±0.2
Aldosterone	2.6±0.3	*1.7±0.1	2.1±0.2
RU/26752	2.6±0.1	2.4±0.3	2.7±0.2
RU/26752 + Aldosterone	2.4±0.3	2.2±0.2	2.4±0.1

Values expressed are mean ± SEM (n=6).

*Significant difference from corresponding value in control untreated animals ($P<0.05$)

TABLE IV

Effect of glucocoritocid agonist, dexamethasone and mineralocorticoid antagonist RU 26752 treatment on fluid intake, urine volume and organ weights.

	control	Dex 1.5mg	RU26752 7.5mg + Dex 1.5 mg	RU26752 15 mg + Dex 1.5 mg
Water Intake (ml/day)	32.8±0.82	*40.75±1.26	*43±1.6	*40.75±1.2
Urine output (ml/24h)	11.85±0.64	*21±0.8	*17±38	*21.7±0.6
Liver weight	14.16±0.67	*10.5±0.25	*11.16±0.5	*11.04±0.23
Kidney weight	3.14±.96	*2.35±.04	*2.5±.08	*2.35±.08
Heart weight	1.29±.05	*0.98±.05	*1.005±.04	*0.945±.026
Thymus weight	0.49±.028	*0.2±.024	*0.198±.016	*0.254±.02
Adrenal weight	.071±.006	*.037±.002	*0.04±.004	*0.036±.0024

Values expressed are mean ± SEM (n=8)

*P<0.05 compared with control.

CHAPTER 3

The Role of the Novel Antimineralocorticoid, Mespirenone, on Adrenocorticosteroid-Induced Hypertension

Abstract

The effects of the aldosterone antagonist mespirenone on the development and maintenance of hypertension in three different experimental models of hypertension were studied. Uninephrectomized saline-drinking male Sprague-Dawley rats injected i.m. with either 0.2 ml olive oil (control), 50 μg aldosterone, 1 mg mespirenone, 50 μg aldosterone plus 500 μg mespirenone or 50 μg aldosterone plus 1 mg mespirenone, each dissolved in 0.2 ml olive oil. Administration of aldosterone alone significantly increased the systolic blood pressure (SBP) from a control value of 114 ± 3.6 to 162 ± 4 by the end of the three-week experimental period. Mespirenone given alone had no effect on SBP. However, mespirenone given in combination with aldosterone reversed the hypertension caused by aldosterone in a dose-dependent manner. Saline consumption and urinary output were slightly increased in aldosterone-treated rats as compared with the other groups but the body weight and organ weights were comparable in all groups. Also microscopic examination of kidney and heart showed no abnormalities. On the other hand mespirenone was unable to prevent the dexamethasone-induced

hypertension. Instead, the blood pressure was slightly increased (169 ± 3.3) when mespirenone was administered simultaneously with dexamethasone as compared to dexamethasone alone (142 ± 3.0). Interestingly, mespirenone potentiated dexamethasone-induced thymic involution and was unable to reverse the body weight loss resulting from dexamethasone treatment. However, the kidney and heart weights were not affected by mespirenone treatment. SHR animals were given two different doses of mespirenone. At the end of the three-week experimental period, rats injected with 2.5 mg mespirenone showed a reduction in SBP (198.3 ± 3.6) compared to control SHR given only olive oil (215.2 ± 2.8) and rats injected with 5 mg mespirenone demonstrated a further reduction in the SBP (190.0 ± 2.6). However, statistical analysis of the data showed no significant difference between SHR treated with mespirenone and control SHR. These results suggest that the *in vivo* administration of mespirenone to rats (i) effectively prevents the aldosterone-induced hypertension and (ii) had no effect on dexamethasone-induced hypertension. (iii) As compared to glucocorticoids, it is suspected that mineralocorticoids may be more involved in the development of hypertension in the SHR.

Introduction

Though different chemical substitutions have been made on the spironolactone molecule in the hope of decreasing its antiandrogenic and progestogenic activities while at the same time increasing its anti-aldosterone potency, no new steroidal anti-mineralocorticoid has been introduced into the therapy of aldosterone-dependent diseases. Studies have shown that the introduction of a 1,2 double bond into the spironolactone molecule leads to a reduction in the affinity for the androgen and progesterone receptors without changing the antimineralocorticoid potency *in vitro* (237). Additional modifications introducing a 15, 16-methylene moiety significantly increased the antimineralocorticoid potency (238). Such a compound with chemical modifications of a 1,2 double bond and 15ß, 16ß methylene ring on the spironolactone molecule has been synthesized by researchers at Schering and it is known as mespirenone (72-acetylthio-15ß, 16ß-methylene-3-oxo-17α-pregna-1,4-diene-21, 17-carbolactone). In animal studies mespirenone exhibited a three-times greater antiandosterone potency and less than 10% of the antiandrogenic activity of spironolactone (238). In these studies, the antimineralocorticoid effect of mespirenone was assessed by its ability to reverse the renal action of aldosterone which is the sodium retaining and potassium diuretic effect.

In humans, Seibert et al. reported that the antimineralocorticoid potency of mespirenone following oral administration was about six times higher than spironolactone (239). From a therapeutic point of view, an increase in antimineralocorticoid potency of a new aldosterone antagonist by itself is desirable. Moreover, *in vivo* studies in humans showed no progestogenic activity

of mespirenone even at pharmacological doses (240). Thus, mespirenone appears to be the aldosterone antagonist that researchers have been searching for. However, up until now the *in vivo* studies involving mespirenone have focused on its natriuretic property and its endocrinological profile. Since one of the most common complications of excess mineralocorticoids is hypertension, it is necessary to study the *in vivo* effect of mespirenone on hypertension in assessing its anti-mineralocorticoid potency. Therefore, for the first time, a long-term study of the antihypertensive effect of mespirenone on three different experimental models of steroid-induced hypertension was conducted.

Materials and Methods

Three experiments were conducted to study the role of mespirenone in steroid-induced hypertension. To initiate aldosterone-salt-induced hypertension, Sprague-Dawley rats were uninephrectomized under ether anesthesia and given 0.9% saline. One week later, the rats were divided into five groups each containing six rats (n=6). They were injected every other day over a period of 18 days. Group I was given 0.2 ml olive oil (control), Group II received 50 μg aldosterone/300g, Group III was treated with 1 mg mespirenone/300g, Group IV with a combination of 50 μg aldosterone and 500 μg mespirenone/300g (10 times the dose of aldosterone), and Group V with a combination of 50 μg aldosterone and 1 mg mespirenone/300 (20 times the dose of aldosterone). Aldosterone and mespirenone doses for these experiments were chosen based on dose-response studies done previously in this laboratory (unpublished data).

To induce dexamethasone hypertension, Sprague-Dawley rats were divided into four groups with eight rats in each group (n=8). Group I was injected with

0.2 ml olive oil/300g, Group II with 1.5 mg dexamethasone/300g, Group III with both 1.5 mg dexamethasone and 15 mg mespirenone/300g (10 times the dose of dexamethasone), and Group IV received 1.5 mg dexamethasone and 30 mg mespirenone/300 (20 times the dose of dexamethasone).

SHR animals were divided into three groups with eight rats in each (n=8). Group I was injected with the vehicle 0.2 ml olive oil, Group II with 2 mg mespirenone/150g and Group III 5 mg mespirenone/150g dissolved in 0.2 ml olive oil.

Baseline systolic blood pressure (SBP) was measured before steroid administration and then once a week thereafter. An average of six to eight blood pressure measurements within 5 mmHg of each other was used as the representative SBP for each animal. The mean body weight was taken over 6 to 8 readings and this represented the body weight of each rat. Fluid consumption and urinary output over a 24-hour period were collected on two consecutive days each week. At the end of each experiment, the rats were anesthetized with carbon dioxide and quickly killed by cervical dislocation. The heart, kidney, liver and thymus were removed and weighed. Histological preparations were done with the heart and kidney tissues for morphological examinations of any possible lesions due to hormone antagonists treatment. Data were analyzed by the two-way analysis of variance followed by the Scheffe test for multiple comparisons.

Results

Results presented in Figure 7 show a very rapid increase in the blood pressure of aldosterone-treated rats over control rats within a week of steroid administration and remained throughout the experimental period. This increase in

the blood pressure was completely prevented by the mineralocorticoid antagonist mespirenone given simultaneously with aldosterone. Five hundred (500) μg of mespirenone given together with 50 μg of aldosterone reduced the SBP from 162 ± 3.4 (aldosterone-treated value) to 126 ± 3.4 and 1 mg mespirenone plus 50 μg reduced it to 116 ± 1.9 which was similar to the control value (114 ± 3.6).

Data presented in Table V indicate no significant change in body weight in all five groups. Although saline consumption was higher in the aldosterone-treated rats, this was followed by a correspondingly higher urinary output. However, the simultaneous administration of mespirenone with aldosterone maintained saline intake and urinary output at the level of the control animals. There was no significant difference in the organ weights among the groups (Table VI). Microscopic examination of the kidney and heart showed no significant lesions. Microscopic changes were limited to minimal focal myocyte fuchsinophilia of the myocardium which is considered to be insignificant (Data not shown).

Results shown in Figure 8 demonstrate a rapid and significant increase in blood pressure within a week of dexamethasone treatment which continued until the end of the experiment. Mespirenone at two different doses given in combination with dexamethasone was unable to prevent the hypertension induced by dexamethasone treatment. Surprisingly, the blood pressure of rats treated with a combination of dexamethasone and mespirenone (160 ± 3.6; 169 ± 3.5) was higher than rats treated with dexamethasone alone (142 ± 2.5) but the difference was not statistically significant. Thus, mespirenone seemed to have potentiated dexamethasone-induced hypertension. As observed in Table VII a three-week dexamethasone treatment significantly decreased the body weight (289.5 ± 9.0) as

compared to controls (372.6 ± 7.9) and mespirenone was unable to prevent this weight loss (291.5 ± 8.0; 300 ± 13.6). Interestingly, simultaneous administration of mespirenone and dexamethasone resulted in complete involution of the thymus while dexamethasone administered alone caused a partial involution of the thymus (Table VII). However, heart and kidney weights were not affected by the treatment and no abnormalities were found in the hearts and kidneys of the treated groups under microscopic examination. Water intake and urinary output were comparable in all groups (Table VIII).

Data presented in Figure 8 demonstrate that mespirenone does not prevent hypertension developed by SHR. Although the SBP of the rats treated with mespirenone at two different doses (198 ± 3.6; 190.5 ± 2.6) was lower compared to control SHR (215.2 ± 2.8), the difference was not statistically significant. There was no significant change in body weight, water intake, urinary output and organ weights between control SHR and mespirenone-treated SHR animals (Table VII).

Discussion

The results obtained show that aldosterone administered to mononephrectomized saline-drinking rats leads to the development of hypertension. This hypertension can be prevented by the simultaneous administration of the potent mineralocorticoid antagonist, mespirenone with aldosterone. To our knowledge, this is the first study to look at the antihypertensive effect of long-term treatment with mespirenone combined with aldosterone. The potency of this aldosterone antagonist can be appreciated when this study is compared with studies involving other mineralocorticoid antagonists. Results in the previous study show that the newly synthesized

antimineralocorticoid RU 26752 by Roussel Uclaf, prevents aldosterone-induced hypertension in Sprague-Dawley rats (241). However, very high doses of RU 26752 were needed to effectively prevent aldosterone-induced hypertension as compared to mespirenone. To antagonize aldosterone-induced hypertension, studies show that an optimum dose of RU 26752 five hundred times more than the dose of aldosterone which caused an increase in the blood pressure was needed, whereas only ten times the dose of the same amount of aldosterone was needed for mespirenone. Studies involving spironolactone show that even a higher dose than RU 26752 was employed to antagonize mineralocorticoid actions *in vivo* (232). In rats, a dose of spironolactone as high as 26.8 mg/kg/hr is administered to counteract aldosterone action *in vivo* (232). In patients with mineralocorticoid-excess diseases, a dose of 200-400 mg/day of spironolactone is given (193). Therefore, the reduction of the therapeutic dose of mespirenone will not only be economically advantageous but will offer less chance of side effects.

Preliminary studies have been done by other researchers to evaluate the natriuretic effect of mespirenone and observed a three-times higher potency in rats (238) and a six-times higher potency in humans (239) as an antimineralocorticoid than spironolactone. Unlike spironolactone, mespirenone has been shown to have no progestational activity after two weeks of treatment in humans (239) and a markedly reduced antiandrogenic activity (238). Thus, the potency of mespirenone as a mineralocorticoid antagonist with reduced endocrinological side effects is well established. However, the possibility of mespirenone possessing some weak mineralocorticoid agonist activity has not been investigated until now. It is well known that steroid antagonists may also possess

some steroid agonist properties and are known as suboptimal antagonists (203). According to these results, rats given mespirenone alone showed a slight increase in the blood pressure over control rats. This is consistent with other steroid antagonists such as RU 486 which was shown by a study in our lab to possess some weak glucocorticoid agonist properties in the early phase of administration (5). However, since the increase in blood pressure of the rats treated with mespirenone alone was not statistically significant, this slight elevation might be due to variabilities in that group and not due to mespirenone treatment.

Surprisingly, the simultaneous administration of mespirenone and dexamethasone caused a complete involution of the thymus. The reason for this observation, (mespirenone potentiating dexamethasone-induced thymic involution) was unexpected and seems to indicate that mespirenone may have some glucocorticoid agonist activity since glucocorticoid-induced thymic involution is known to be a Type II-mediated mechanism. But this was proved not to be the case as results from another experiment in our lab showed that glucocorticoid receptors were not down regulated by mespirenone. Thymus weight was not affected by mespirenone treatment in the other groups and microscopic examination of the kidney and heart tissues showed no abnormal lesions due to mespirenone treatment.

Thus, mespirenone is a novel mineralocorticoid antagonist which might offer clinical advantages over spironolactone in the treatment of mineralocorticoid exces diseases such as mineralocorticoid-induced hypertension with far less deleterious endocrinological side effects.

Figure 7: Effect of the mineralocorticoid agonist, aldosterone and mineralocorticoid antagonist, mespirenone, treatment on SBP of Sprague-Dawley rats. Each point represents the mean ± *P<0.05 compared with control rats.

FIGURE 7

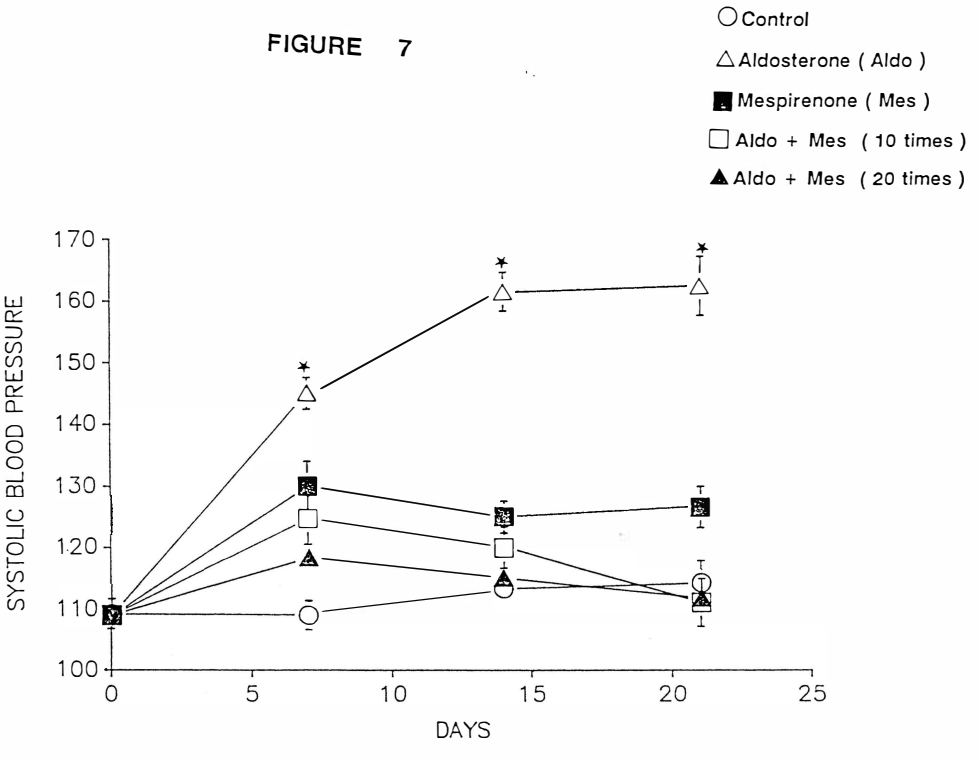
Control
Aldosterone (Aldo)
Mespirenone (Mes)
Aldo + Mes (10 times)
Aldo + Mes (20 times)
SYSTOLIC BLOOD PRESSURE
170
160
150
140
130
120
110
100
0
5
10
15
20
25
DAYS

Figure 8: Effect of glucocortcoid agonist, dexamethasone, and the mineralocorticoid antagonist, mespirenone, treatment on SBP. Each point represents mean ± SEM of eight male Sprague-Dawley rats. *P<0.05 compared with controls.

FIGURE 8

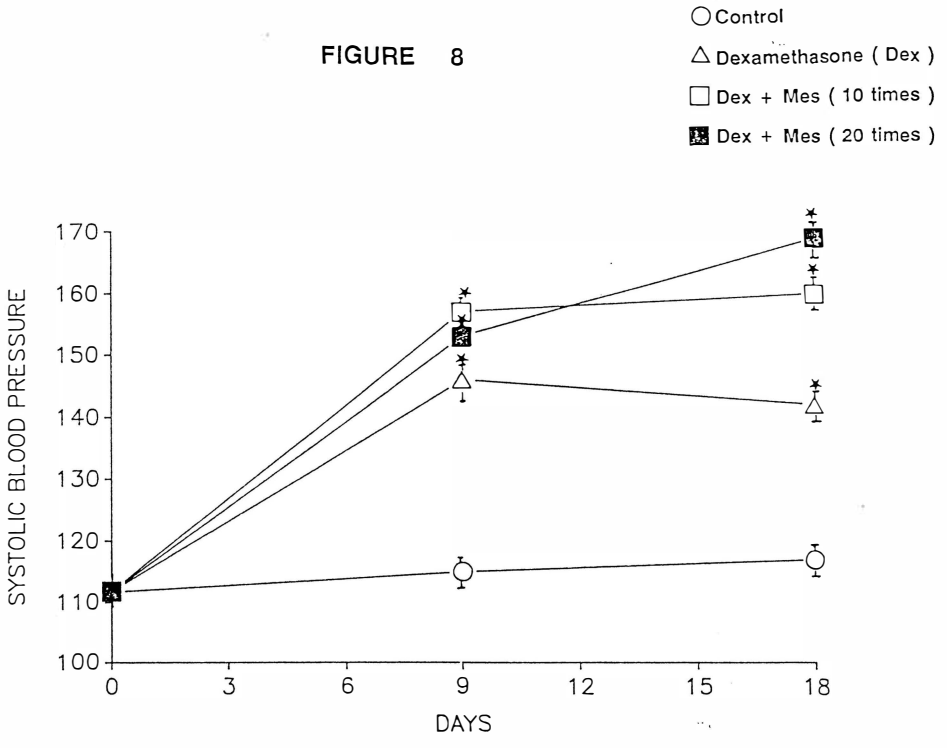
Control
Dexamethasone (Dex)
Dex + Mes (10 times)
Dex + Mes (20 times)
SYSTOLIC BLOOD PRESSURE
170
160
150
140
130
120
110
100
0
3
6
9
12
15
18
DAYS

Figure 9: Effect of mespirenone treatment on systolic blood pressure of spontaneously hypertensive rats (SHR). Each point represents means ± SEM of eight rats.

FIGURE 9

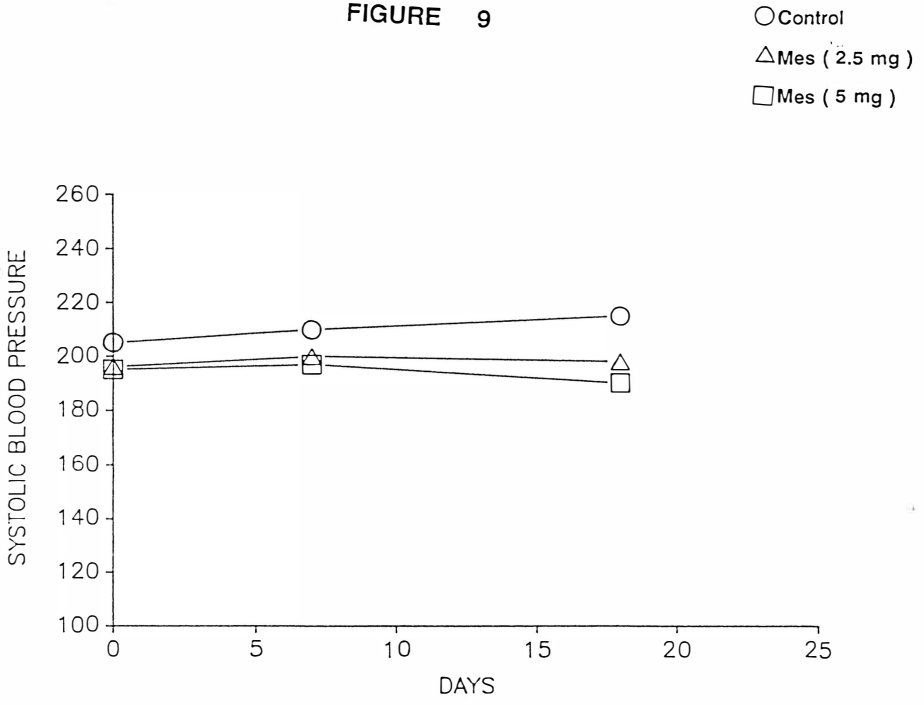
Control
Mes (2.5 mg)
Mes (5 mg)
SYSTOLIC BLOOD PRESSURE
260
240
220
200
180
160
140
120
100
0
5
10
15
20
25
DAYS

TABLE V

Body weight, saline consumption and urine output in rats treated with aldosterone and aldosterone plus mespirenone.

	Control	Aldo	Meso	Aldo + 10 x Meso	Aldo + 20 x Meso
Body weight (gms)					
day 0	289 ± 5.6	284 ± 4.9	273 ± 4.7	281 ± 2.5	282 ± 3.9
day 7	343 ± 7.8	303 ± 13.7	299 ± 8.8	334 ± 5.4	308 ± 9.4
day 14	341 ± 3.5	322 ± 5.2	327 ± 2.2	369 ± 4.2	314 ± 10.0
day 21	405 ± 6.9	388 ± 5.9	380 ± 7.2	317 ± 4.5	362 ± 9.5
Urine out put (ml/24hr)	25 ± 1·8	* 34 ± 1·4	24 ± 2·05	28·0 ± 2·4	23·0 ± 3·3
Saline consumption (ml/24hr)	36·41 ± 1·6	* 45·9 ± 2·4	48·3 ± 3·4	42·6 ± 2·1	40·3 ± 3·1

TABLE VI

Effect of glucocorticoid agonist, dexamethasone and mineralocorticoid antagonist, mespirenone treatment on body weight and organ weights.

	Treatment Groups			
	Control	Dex	Dex + (15mg MSP)	Dex + 30mg MSP
Body Weight (gm)				
Day 0	316·8 ± 4·5	334·8 ± 6·3	330·3 ± 3·9	330·6 ± 3·3
Day 7	347·5 ± 3·9	299·6 ± 6·7	303·2 ± 6·5	290·5 ± 9·0
Day 17	372·6 ± 7·9	289·5 ± 9·0*	291·5 ± 8·0*	300 ± 13·6**
Water intake (ml/24hr)	39.5 ± 5.1	41.5 ± 4.6	37.4 ± 3.8	42.0 ± 2.4
Urine volume (ml/24hr)	15.6 ± 2.6	13.3 ± 2.3	15.8 ± 2.1	15.5 ± 1.7
Thymus , mg	340mg ± 50	87 ± 20	---	---
Heart, mg	1,180 ± 80	1,000 ± 30	1,050 ± 30	1,040 ± 40
Kidney, mg	2,940 ± 250	2,800 ± 110	2,670 ± 100	2,980 ± 110

Table VII

Effect of Mespirenone Treatment on Body Weight, Water Intake, Urine Output and Organ Weight of Spontaneously Hypertensive rat (SHR)

Body Weight (g)	Control (2.5 mg)	Mespirenone	Mespirenone (5 mg)
Day 7	337 ± 4.3	336 ± 2.5	339 ± 6.2
Day 14	343 ± 5.7	337 ± 4.1	334 ± 8.5
Day 21	361 ± 4.3	351 ± 3.7	345 ± 7.5
Saline Intake (ml/day)	28.3 ± 2.1	23.0 ± 1.8	32.1 ± 3.2
Liver weight (g)	13.5 ± 0.4	14.3 ± 0.2	15.1 ± 1.1
Kidney weight (g)	2.8 ± 0.07	3.0 ± 0.04	3.1 ± .17
Heart weight (g)	1.5 ± 0.04	1.5 ± 0.02	1.5 ± 0.12

CONCLUSION

From the results presented in this study, RU 486 seems to be a potent glucorticoid antagonist without any effect on mineralocorticoid hypertension. Thus, RU 486 may have potential clinical use in the treatment of glucocorticoid-induced hypertension, the most common complication associated with glucocorticod excess diseases such as Cushing's Syndrome.

Although the mineralocorticoid antagonist spironolactone, is clinically used in the treatment of mineralocorticoid excess diseases, it is limited by numerous adverse endocrinological side effects. Accoridng to the results presented here, mespirenone appears to be a better substitute for spironolactone than RU 26752 in the treatment of mineralocorticoid excess diseases. Not only was a far less amount needed to antagonize the *in vivo* actions of aldosterone but also, mespirenone has been shown by others to have negligible side effects associated with spironolactone.

This work has established these three novel steroid hormone antagonists as potent and specific *in vivo*. Apart from their potential therapeutic use as discussed above, steroid hormone antagonists can be used as tools to probe the molecular mechanism of action of steroid hormones about which little is known. At this point, it is known that the steroid hormones exert their biological effects by

regulating gene expression in the cells of the target tissues. Due to the recent availability of cDNA for both the glucocorticoid and mineralocorticoid receptors, work has begun in our lab to measure the glucocorticoid receptor and mineralocroticoid receptor expression during steroid-induced hypertension and during the inhibition of hypertension by these antagonists. This is consistent with my objective to explore the molecular mechanism by which steroid hormones induce hypertension as more cDNA probes of other steroid-hormone target genes become available in the future.

APPENDIX

APPENDIX A

Table VIII

Effect of the Mineralocorticoid Agonist, DOCA, and the Glucocorticoid Antagonist, RU 486, on Systolic Blood Pressure

Treatment Groups	Week of Treatment First	Second	Third
Control	119.0 ± 3.6	127.5 ± 3.5	119.3 ± 4.1
1 mg DOCA	120.5 ± 3.6	170 ± 2.8	173.6 ± 1.6
1 mg DOCA + 10 mg RU 486	121.7 ± 2.4	173.6 ± 1.6	152 ± 3.6

APPENDIX B

Table IX

Effect of the Glucocorticoid Antagonist, RU 486 Treatment on the Systolic Blood Pressure of Spontaneously Hypertensive Rats (SHR)

Treatment Groups	Week of Treatment First	Second	Third
Control	145 ± 2.1	147.5 ± 1.7	150 ± 1.8
1 mg RU 486	140.6 ± 1.4	155 ± 2.9	160 ± 2.5
5 mg RU 486	145.6 ± 2.4	160.9 ± 3.1	172.5 ± 2.5

Values expressed are mean ± SEM (n=8)
*($p<0.05$) compared with control.

APPENDIX C

Table X

Effect of the Mineralocorticoid Agonist Aldosterone and the Mineralocorticoid Antagonist, Mespirenone Treatment on Systolic Blood Pressure

Treatment Groups	0	First	Second	Third
Control	109.2 ± 2.4	110.3 ± 1.9	114.2 ± 3.6	115 ± 2.6
Aldosterone (Aldo)	108.7 ± 3.1	*145 ± 2.6	*161.6 ± 5.2	*162.5 ± 4.8
Mespirenone (Mes)	110.0 ± 2.7	124.7 ± 4.2	120.0 ± 3.3	114.0 ± 3.9
Aldo + 10 Mes	109.0 ± 1.9	130.1 ± 4.0	125.0 ± 2.6	126.6 ± 3.4
Aldo + 20 Mes	108.2 ± 3.3	118.3 ± 1.9	115.0 ± 1.6	110.7 ± 1.9

Values expressed as mean ± SEM (n=6).
*($p<0.05$) compared to control untreated animals.

APPENDIX D

Table XI

Effect of the Glucocorticoid Agonist, Dexamethasone, and the Mineralocorticoid Agonist, RU 26752 Treatment on Systolic Blood Pressure

Treatment Groups	0	Week of Treatment First	Second
Control	115.8 ± 3.6	116.3 ± 4.2	118.8 ± 4.3
Dexamethasone (Dex)	116.0 ± 2.8	*153.1 ± 2.6	*158.2 ± 4.2
Dex + RU 26752 (7.5 mg)	116.8 ± 2.9	*146.3 ± 2.4	*162.5 ± 3.6
Dex + RU 26752 (15 mg)	115.6 ± 2.5	*154.4 ± 2.9	*151.9 ± 2.9

Values expressed are mean ± SEM (n=8)
*($P<0.05$) compared to control untreated animals.

APPENDIX E

Table XII

Effect of the Mineralocorticoid Agonist Aldosterone and the Mineralocorticoid Antagonist, Mespirenone Treatment on Systolic Blood Pressure

Treatment Groups	0	First	Second	Third
Control	109.2 ± 2.4	110.3 ± 1.9	114.2 ± 3.6	115 ± 2.6
Aldosterone (Aldo)	108.7 ± 3.1	*145 ± 2.6	*161.6 ± 5.2	*162.5 ± 4.8
Mespirenone (Mes)	110.0 ± 2.7	124.7 ± 4.2	120.0 ± 3.3	114.0 ± 3.9
Aldo + 10 Mes	109.0 ± 1.9	130.1 ± 4.0	125.0 ± 2.6	126.6 ± 3.4
Aldo + 20 Mes	108.2 ± 3.3	118.3 ± 1.9	115.0 ± 1.6	110.7 ± 1.9

Values expressed as mean ± SEM (n=6).
*($p<0.05$) compared to control untreated animals.

APPENDIX F

Table XIII

Effect of Glucocorticoid Agonist, Dexamethasone, and the Mineralocorticoid Antagonist, Mespirenone (Mes), Treatment on Systolic Blood Pressure

Treatment Groups	Week of Treatment 0	First	Second
Control	110 ± 2.1	115.0 ± 3.2	117.0 ± 3.3
Dexamethasone (Dex)	110.9 ± 3.6	*146.0 ± 4.2	*142.1 ± 2.5
Dex + Mes (15 mg)	111.8 ± 4.2	*157.0 ± 3.2	*160.2 ± 3.6
Dex + Mes (30 mg)	111.0 ± 3.0	*153.2 ± 2.9	*169.0 ± 3.5

Values expressed are mean ± SEM (n=8).
*($P<0.05$) compared to control untreated animals.

APPENDIX G

Table XIV

Effect of the Mineralocorticoid Antagonist, Mespirenone, Treatment on Systolic Blood Pressure of Spontaneously Hypertensive Rats (SHR)

Treatment Groups	Week of Treatment 0	First	Second
Control	205.0 ± 3.5	210.1 ± 3.0	215.2 ± 2.8
Mespirenone (2.5 mg)	196.2 ± 1.9	200.0 ± 2.2	198.3 ± 3.6
Mespirenone (5 mg)	195.0 ± 2.8	197.2 ± 3.8	190.0 ± 2.6

Values expressed are mean ± SEM (n=8).

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LITERATURE CITED

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