Effects of the Glucocorticoid II Receptor Antagonist Mifepristone on Hypertension in the Obese Zucker Rat

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Accepted for publication April 8, 1997

ABSTRACT
We have investigated the possible involvement of endogenous corticosteroids in the maintenance of hypertension in aged lean and obese Zucker rats using the type II corticosteroid antagonist mifepristone. At 8 mo of age, the start of the study, obese Zuckers had been hypertensive for at least 2 mo (systolic blood pressure; 153 ± 4 vs. 136 ± 5 mmHg; n = 8–9; P < .05) and were hyperinsulinemic (756 ± 98 vs. 193 ± 61 μU · ml⁻¹) and hypercorticosteronemic (524 ± 83 vs. 260 ± 97 ng · ml⁻¹) compared to their lean littermates. There were no differences in plasma renin activity between lean and obese animals and plasma renin activity was unaffected by any treatment. Oral treatment of obese rats with mifepristone (40.0 mg · kg⁻¹ · day⁻¹ for 9 days) resulted in a gradual reduction in SBP to lean levels by day 9. Mifepristone treatment did not affect plasma insulin or corticosterone levels but resulted in a significant reduction in plasma aldosterone concentration. Mifepristone was without significant effect on systolic blood pressure in lean rats. Oral treatment of lean rats with corticosterone-21-acetate (3.0 mg · kg⁻¹ · day⁻¹ for 9 days) resulted in a rise in systolic blood pressure to levels similar to obese Zuckers after 9 days. Plasma insulin levels were unchanged but corticosterone immunoreactivity was significantly reduced. Plasma aldosterone levels were increased from 564 ± 3 to 802 ± 68 pg · ml⁻¹. Our data suggest that raised glucocorticoids and aldosterone may be factors contributing to hypertension in obesity.

It is well established that there is a strong clinical association between insulin resistance, obesity and hypertension (DeFronzo, 1990; Haefner et al., 1992; Wajchenberg et al., 1994; Ferrannini et al., 1990). However, despite this epidemiological evidence and the recognition that patients with essential hypertension are insulin resistant (Ferrannini et al., 1990; DeFronzo, 1990) the underlying pathogenic link between hypertension, obesity and insulin resistance remains uncertain. Nevertheless, there is clear evidence to suggest that hyperinsulinemia is predictive of and causal in the development of noninsulin-dependent diabetes and hypertension (DeFronzo, 1990; Wajchenberg et al., 1994). Furthermore there are several mechanisms that could explain this association, including insulin-stimulated sodium reabsorption in the kidney, stimulation of the sympathetic nervous system, alteration of membrane ion transport, altered vascular reactivity or impaired insulin dependent arteriolar vasodilatation (DeFronzo, 1990; Daly and Landsberg, 1991; Corry and Tuck, 1996).

Obesity, hyperinsulinemia and hyperlipidemia also characterize the genetic defect in the obese Zucker rat (Bray and York, 1979) and in this regard it manifests all the metabolic abnormalities that characterize the insulin resistance Syndrome X in man (Reaven, 1988; Wajchenberg et al., 1994). In addition to these perturbations in metabolism, arterial pressure has been shown to be higher in obese rats compared to their lean littermates after 6 mo of age (Kurtz et al., 1989; Kasiskie et al., 1992; Turner et al., 1995). The mechanism for the elevation of blood pressure, however, is unclear, particularly because sympathetic drive (Levin et al., 1983) and plasma renin activity (Harker et al., 1993) are reported to be reduced in obese Zucker rats, but it has been proposed to be secondary to renal injury or increased renal sodium reabsorption (Kasiskie et al., 1992).

Recent evidence suggests that the primary cause of obesity in the falfa Zucker rat is a mutation in the leptin receptor (Philips et al., 1996). Nevertheless, high corticosterone levels are thought to play an important role in the development and maintenance of the obesity syndrome in the Zucker rat. Thus expression of the obese phenotype can be prevented in weanling or partially reversed in postweanling obese Zucker rats by adrenalectomy (Fletcher, 1986; Freedman et al., 1986; Castonguay, 1991) and restored in adrenalectomized obese Zucker rats by corticosterone replacement (Fletcher, 1986; Freedman et al., 1986; Castonguay, 1991). Similarly, mifepristone, a type II glucocorticoid receptor antagonist (Brogden et al., 1993), has been reported to reduce obesity and hyperphagia and to ameliorate the hyperinsulinemia of

ABBREVIATIONS: SBP, systolic blood pressure; PRA, plasma renin activity; ANOVA, analysis of variance.
young obese rats mimicking the effect of adrenalectomy (Lan-
gley and York, 1990).

It is well established that both glucocorticoids and miner-
alocorticoids increase blood pressure and that clinical condi-
tions such as Cushing’s syndrome characterized by raised
glucocorticoid levels are associated with hypertension (Walk-
er and Edwards, 1994). In addition, administration of corti-
osterone or selective type II glucocorticoid agonists to nor-
mal rats induces a reversible hypertension that is prevented
or reversed by inhibition of type II, but not type I, glucocor-
ticoid receptors (Grunfeld et al., 1985). Furthermore, sodium-
dependent hypertension in humans with glucocorticoid ex-
cess (Cushing’s syndrome) can be controlled by mifepristone
but not the type I antagonist spironolactone (Mantero and
Boscaro, 1992).

There is a general lack of information regarding the patho-
genesis of hypertension associated with obesity but it has
been suggested that the Zucker rat might be a suitable ani-
mal model of obesity and hypertension (Kurtz et al., 1989). In
view of the importance of glucocorticoids to the develop-
ment and maintenance of the obese phenotype in Zucker rats and
their connection with essential hypertension in man the aim
of our study was to investigate the involvement of glucocor-
ticoids in hypertension in aged obese Zucker rats. We have
used the glucocorticoid II receptor antagonist mifepristone to
explore the contribution of endogenous corticosterone to the
maintenance of hypertension in obese Zucker rats and com-
pared this to the effects of corticosterone-21-acetate on blood
pressure in lean animals.

Methods and Materials

Adult male obese Zucker rats and their lean littermates were
obtained from Harlan Olac (Bicester, Oxfordshire, U.K.) and fed
RM1 diet (SDS, Witham, U.K.) ad libitum during the run-up to
dosing and allowed free access to water at all times. All experi-
ments were approved by the Procedures Review Panel of SmithKline
Beecham Pharmaceuticals U.K. and complied with the Guidance on
the Operation of the Animals (Scientific Procedures) Act 1986.

Blood pressure measurement. In view of excessive lipid depo-
sition in indwelling cannula and poor surgical wound healing in
obese Zucker rats (personal observations) blood pressure was mea-
sured noninvasively using a tail cuff plethysmographic method
(Apollo model 179; ITTC Life Science, Woodland Hills, CA) that has
been previously validated (Bunag and Butterfield, 1984; Bunag,
1984). The animals were acclimatized to an ambient temperature of
29 to 31°C for 15 min before blood pressure determination and values
were taken from the mean of at least three recordings per animal.

Study design. Rats were placed in perspex restraining tubes and
housed in an incubator maintained at 29 to 32°C. After a 15-min
equilibration period, blood pressure was measured using an inflat-
able cuff and pulse sensor, placed around the tail, coupled to a model
679 semiautomatic blood pressure system (ITTC Inc., Woodland
Hills, CA). The inflated cuff pressure was 275 mmHg and pressure
was released at a rate of 500 mmHg min⁻¹. Systolic blood pressure
was calculated as the mean of at least three readings. Blood pressure
was monitored monthly, from 5 mo of age, until SBP in obese rats
was significantly greater than their lean littermates for 2 consecu-
tive mo. Drug treatment was initiated when the animals were 8 mo
of age. Animals were randomly assigned to a control group or to a
control group. All animals received drug vehicle (propylene glycol,
2.0 ml · kg⁻¹ day⁻¹) by gavage for 14 days starting at day 1. Blood
pressure was measured on three further occasions on days 3, 8 and
10 to establish a baseline level of SBP. One obese and one lean group
then received mifepristone (a gift from Roussel-UCLAF, Paris,
France; 40.0 mg · kg⁻¹ day⁻¹) a second lean group received cortico-
terone-21-acetate (3.0 mg · kg⁻¹ day⁻¹); control animals (lean and
obese) remained on vehicle and dosing continued once daily for
another 9 days. Blood pressure measurements were made 2, 4 and
9 days after commencement of dosing.

Because it was anticipated that drug treatment would affect food
consumption (Langley and York, 1990), control groups were pair fed
with their corresponding drug-treated animals. On the last day ani-
mals were fasted overnight (15 hr) and anesthetized with pentobar-
bionate sodium the next morning. Terminal blood samples were taken
from the vena cava into heparinized syringes after both renal arter-
ies and veins were clamped off to prevent hemorrhage release of
renin.

Analysis. Whole blood was centrifuged at 3000 rpm for 10 min at
4°C. Plasma was aspirated and aliquots stored at −20°C before
measurement of plasma hormones. Commercially available radioim-
nunoassay kits were used to measure plasma insulin (Amersham
International plc, Amersham, U.K.), aldosterone (DPC Diagnostics,
Caernarfon, U.K.), corticosterone (Amersham International plc) and
plasma renin activity (New England Nuclear, Stevenage, U.K.). Glu-
cose levels were determined by the glucose oxidase method from
hemolyzed whole blood.

Statistical analysis. Results are expressed mean ± S.E. Statis-
tical analysis between groups was performed by one-way ANOVA.
Where significant between-group variation was observed, Dunnett’s test
was performed for multiple comparisons (Wallenstein et al., 1980) was
performed to identify the source of variance. All statistical analyses
were performed using SAS Research Statistic Application program,
version 1.4 (SAS Institute Inc., Carey, NC). P < .05 (95% level) were
considered significant.

Results

Significant differences in systolic blood pressure between
obese Zucker rats and their lean littermates were observed
from 6 mo of age (table 1). Obese animals were also signifi-
cantly heavier and were hyperphagic compared to their lean
littermates at the start of the study at 8 mo of age (table 1).
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hyperphagic compared to their lean littermates at 8 mo of age (table 1).
At the end of the study period, obese Zucker rats receiving
vehicle gained 13 g body weight compared to a 25 g loss of
weight in the mifepristone treated obese animals (table 2).

| TABLE 1 |
| Base-line data for aged Zucker rats before dosing |
| SBP (mmHg) | SBP (mmHg) | SBP (mmHg) | SBP (mmHg) | Body Weight (g) | Food Intake (g · 24 hr⁻¹) |
| Age at measurement | 5 mo | 6 mo | 7 mo | 8 mo | 8 mo | 8 mo |
| Obese Zucker vehicle | 146 ± 5 | 171 ± 5* | 166 ± 4.7* | 160 ± 5* | 660 ± 37* | 24.4 ± 0.4* |
| Obese Zucker mifepristone | 143 ± 4 | 154 ± 5* | 153 ± 6.9* | 160 ± 4* | 649 ± 34* | 23.6 ± 0.3* |
| Lean Zucker vehicle | 139 ± 6 | 138 ± 5 | 130 ± 3 | 138 ± 3 | 431 ± 14 | 19.1 ± 0.3 |
| Lean Zucker mifepristone | 140 ± 4 | 142 ± 8 | 141 ± 5 | 142 ± 6 | 423 ± 15 | 18.4 ± 0.4 |
| Lean Zucker corticosterone-21-acetate | 132 ± 8 | 123 ± 4 | 125 ± 8 | 132 ± 7 | 413 ± 23 | 18.7 ± 0.3 |

Values are mean ± S.E. for six to nine animals per group.
* P < .05 compared to lean Zucker vehicle group.
Neither mifepristone nor corticosterone-21-acetate treatment significantly affected body weight compared to vehicle treated leans in this study (table 2).

**Effects of mifepristone in lean and obese Zucker rat.**
Before dosing, SBP in obese rats was significantly higher than in their lean littermates (table 1). During administration of mifepristone (40.0 mg \( \cdot \) kg\(^{-1} \) day\(^{-1} \) p.o.), SBP gradually decreased to a level of 134 ± 4 mmHg after 9 days of oral dosing although SBP in vehicle-treated obese Zucker rats was unchanged from baseline at 153 ± 4 mmHg and remained significantly higher than vehicle treated lean rats (132 ± 5 mmHg; fig. 1). In lean rats, SBP was not significantly affected by mifepristone (fig. 2).

At the termination of the experiment obese Zucker rats were hypercorticosteronemic and hyperinsulinemic with respect to their lean littermates and there was no evidence for stimulation of the renin-angiotensin-system as judged by PRA or plasma K\(^+\) levels (table 2). Once daily oral dosing for 9 days with mifepristone (40.0 mg \( \cdot \) kg\(^{-1} \) day\(^{-1} \)) did not significantly modify corticosteroid levels, insulin levels or PRA in either the lean or obese aged Zucker rat (table 2).

In our study obese rats were slightly, but not significantly, hyperglycemic with respect to their lean controls. Treatment with mifepristone (40.0 mg \( \cdot \) kg\(^{-1} \) day\(^{-1} \)) was without effect on plasma glucose levels in either the obese or the lean rat (table 2).

Plasma aldosterone concentration was 565 ± 31 ng \( \cdot \) ml\(^{-1} \) and 692 ± 106 ng \( \cdot \) ml\(^{-1} \) in lean and obese Zucker rats respectively (table 2). Mifepristone (40.0 mg \( \cdot \) kg\(^{-1} \) day\(^{-1} \)) elicited a marked and significant \((P < .05)\) reduction in plasma aldosterone in the obese to 362 ± 53 ng \( \cdot \) ml\(^{-1} \) but not the lean, Zucker rat (table 2).

**Effect of corticosterone 21 acetate in lean Zucker rats.** Administration of corticosterone-21-acetate (3.0 mg \( \cdot \) kg\(^{-1} \) day\(^{-1} \)) in both lean and obese Zucker rats was without effect on plasma hormone levels (table 2). One day after dosing with corticosterone-21-acetate (3.0 mg \( \cdot \) kg\(^{-1} \) day\(^{-1} \) p.o., n = 7–9) and lean Zucker rats treated with propylene glycol \( (2.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \text{ p.o., } n=9) \) drug treatment phase indicated by solid box. Values are mean ± S.E. *p < .05 vs. obese vehicle.
kg\(^{-1}\) day\(^{-1}\)) to lean Zucker rats induced a gradual rise in SBP from 133 ± 3 mmHg at the start of dosing to 150 ± 15 mmHg after 9 days of once daily oral dosing (fig. 3). SBP in vehicle-treated leans remained significantly lower than their obese littersmates tending to decline slightly during the course of the experiment (fig. 3).

At the termination of the experiment, plasma corticosterone immunoreactivity was reduced in lean rats treated with corticosterone-21-acetate compared to vehicle treated leans (table 2). Plasma insulin levels and PRA were unchanged in the corticosterone-21-acetate (3.0 mg \(\cdot kg^{-1}\) day\(^{-1}\)) group (table 2). Nine days treatment with corticosterone-21-acetate (3.0 mg \(\cdot kg^{-1}\) day\(^{-1}\)) significantly (\(P < .05\)) increased plasma aldosterone concentration compared to their vehicle treated counterparts (table 2).

### Discussion

Numerous studies have shown that glucocorticoids are crucial to the development of obesity in animal models (Bray and York, 1979; Bray et al., 1990) and for the full expression of the metabolic disorders which characterize the obese Zucker rat (Freedman et al., 1986; Langley and York, 1990; Castonguay, 1991). In man, abdominal obesity and increased visceral fat mass are associated with hypertension and it has been suggested that the link between the two is insulin resistance and hyperinsulinemia (Kissebah and Krakower, 1994). Previous studies in obese Zucker rats have shown that both adrenalectomy and the antiglucocorticoid mifepristone inhibits the development of obesity and ameliorates the attendant hyperinsulinemia and hyperlipidemia (Freedman et al., 1986; Langley and York, 1990; Alarrayed et al., 1992). In our study we have demonstrated that from 6 mo of age obese rats are hypertensive compared to their lean littersmates and that this increase in systolic blood pressure is reversed by treatment with mifepristone. Mifepristone is a type II glucocorticoid receptor antagonist that does not bind to the type I mineralocorticoid receptor or to aldosterone receptors, but that also is an antagonist at progesterone and androgen receptors (Brogden et al., 1993). The similarity between the effects of mifepristone and adrenalectomy on obesity in the obese Zucker rat and in dietary models, suggests that it is the type II receptor rather than progesterone receptors that regulates the development of obesity (Langley and York, 1990; Okada et al., 1992). Similarly in our experiments our observation that the blood pressure of lean Zuckers could be elevated to levels seen in obese animals by treatment with corticosterone-21-acetate together with its reduction in obese animals by mifepristone, lends support to the hypothesis that it is also the type II receptor that is involved in hypertension in the obese animal.

The mechanisms mediating hypertension in the obese Zucker is unclear. Previous studies (Levin et al., 1983; Kasiske et al., 1992) indicate that the obese Zucker has diminished sympathetic nervous system activity and plasma renin activity is not raised (this study) or is reduced (Harker et al., 1993). Nevertheless, both angiotensin-converting enzyme inhibitors and angiotensin II antagonists reduce blood pressure in obese Zucker rats (Schnitz et al., 1992; Crary et al., 1995). In this regard it is interesting to note that mifepristone has been reported to inhibit both glucocorticoid-induced hypertension in rats and angiotensinogen synthesis (Agarwal et al., 1987).

In NIDDM subjects there is a positive correlation between insulin levels and blood pressure (Corry and Tuck, 1996) and there are a number of mechanisms by which hyperinsulinemia might mediate hypertension (Corry and Tuck, 1996). Furthermore agents that improve insulin responsiveness
such as the insulin-sensitizing thiazolidinediones, CS 045 and ciglitazone, are reported to be antihypertensive in the obese Zucker rat (Yoshioka et al., 1993; Pershad Singh et al., 1993). The effects of mifepristone on blood pressure in our study, however, appeared independent of hyperinsulinemia as judged by the unchanged fasting plasma insulin levels.

A surprising finding of our study was that plasma aldosterone levels were greater in obese animals than their lean counterparts and were reduced to lean levels by mifepristone. The mechanism by which mifepristone reduced, and corticosterone-21-acetate increased, plasma aldosterone levels, in the absence of changes in PRA is uncertain but suggests a mechanism independent of renal renin secretion. In support of this, weight gain in the dog has been reported to be associated with an increase in plasma aldosterone without changes in plasma renin activity (Rocchi et al., 1989) and increased plasma aldosterone concentrations despite normal plasma renin activity has been reported in obese man (Ljutic et al., 1995). Adrenal renin and angiotensin II levels are reported to be high (Inagami et al., 1989; Philips et al., 1993) and it is known that there is an intrinsic renin-angiotensin system within the adrenal cortex that regulates aldosterone secretion (Gupta et al., 1995; Mulrow and Franco-Saenz, 1996). It is hypothesized therefore that the adrenal renin-angiotensin system provides a local mechanism for the regulation of aldosterone secretion (Mulrow and Franco-Saenz, 1996). Indeed it is known that ACTH stimulation of aldosterone production is reduced by ACE inhibitors (Ramirez et al., 1988). Our current data suggest that the renin-angiotensin system in the adrenals may also be regulated by glucocorticoids. It is possible that glucocorticoids up-regulate expression of the angiotensin AT1 receptor as is reported in the vasculature (Provencher et al., 1995) increasing the responsiveness of the aldosterone secreting cells to locally produced angiotensin II and to ACTH. In addition an interaction between glucocorticoids and the adrenal renin-angiotensin system may provide a possible mechanism to explain the antihypertensive effects of both the antiguocorticoid mifepristone and ACE inhibitors or angiotensin II antagonists in obese Zucker rats. This hypothesis places aldosterone as an important contributor to elevated blood pressure in the obese Zucker and in this regard it is interesting to note that resting plasma aldosterone concentrations are also reported to be one of the contributing factors responsible for the elevation of blood pressure in obese subjects (Ljutic et al., 1995). Nevertheless hypertension in the obese animal is likely to be a multifactorial process and other factors such as inhibition of the effects of glucocorticoids on vascular tone and responsiveness may be involved in the antihypertensive effects of mifepristone in these experiments.

Our data and that of others support a connection between raised circulating glucocorticoid levels, obesity and hypertension in Zucker rats. Although there is little question about the association between abdominal obesity and increased blood pressure and insulin resistance in man (Kissebah and Krakower, 1994) their association with elevated cortisol secretion is less certain. However, there is increasing evidence that abdominal fat deposition is linked to hyperreactivity of the hypothalamic-pituitary-adrenal axis (Marin et al., 1992; Pasquali et al., 1993) and both cortisol and aldosterone secretion are reported to be elevated in obese subjects (Marin et al., 1992; Ljutic et al., 1995). Although there is strong evidence to connect increased hypothalamic-pituitary-adrenal axis activity with obesity and insulin resistance (Kissebah and Krakower, 1994), our data are the first to show that the associated hypertension may also be a consequence of the dysregulation of adrenal steroid secretion.

We conclude that corticosterone contributes to the maintenance of hypertension in the obese Zucker rat through mechanisms that might be secondary to increased aldosterone secretion. Our data suggest that raised glucocorticoids and aldosterone may be factors contributing to hypertension in obesity.

References


