Role of vasoconstrictor systems in experimental glucocorticoid-hypertension in rats

BERNARD WAEBER, HARALAMBOS GAVRAS, MARGARET R. BRESNAHAN, IRENE GAVRAS AND H. R. BRUNNER

Department of Medicine, Boston University Medical Center, Boston, MA, U.S.A.

(Received 21 June 1982/4 January 1983; accepted 3 March 1983)

Summary

1. The blood pressure effect of SKF 64139, a phenylethanolamine N-methyltransferase (PNMT) inhibitor acting both in the central and peripheral nervous system, was studied in conscious normotensive and glucocorticoid-hypertensive rats maintained for 2 weeks on a salt-deficient diet. On the day of the experiment, mean blood pressure was 119.2 ± 1.1 mmHg (mean ± SEM) in the normotensive and 143.2 ± 1.9 mmHg (P < 0.001) in the hypertensive rats. In all animals, the blood pressure was followed for 4 h after oral gavage by either SKF 64139 (50 mg/kg) or its vehicle (5% glucose solution).

2. In the vehicle-treated rats, no change in blood pressure occurred. However, after SKF 64139 administration, a 17.9 ± 3.8 mmHg (P < 0.001) blood pressure fall was observed in the hypertensive rats whereas normotensive animals did not decrease their pressure.

3. Plasma catecholamines were determined in all these rats at the end of the observation period. No significant difference in plasma noradrenaline and adrenaline levels was observed between normotensive and hypertensive as well as vehicle- and SKF 64139-treated rats, though both catecholamines tended to be higher after administration of the PNMT inhibitor.

4. In two additional groups of glucocorticoid-hypertensive rats, the blood pressure effects of an infusion of saralasin, a competitive antagonist of angiotensin II, or of a bolus injection of a vasopressin analogue antagonizing the pressor action of vasopressin, were investigated over a 20 min period.

The vasopressin antagonist had no blood pressure effect whereas saralasin decreased mean blood pressure from 144 ± 1.9 to 136 ± 3.2 mmHg (P < 0.005).

5. These data suggest that the sympathetic nervous system is the main vasoconstrictor system involved in the maintenance of established glucocorticoid hypertension. Since SKF 64139 is also known to inhibit to some degree α-adrenoceptors, monoamine oxidase and neuronal uptake processes, it remains unclear, however, whether the blood pressure-lowering effect of this PNMT inhibitor can be attributed to central blockade of adrenaline synthesis.

Key words: catecholamines, glucocorticoid-hypertension, PNMT inhibitor, saralasin, vasopressin antagonist.

Abbreviations: AVP, arginine-vasopressin.

Introduction

Experimental glucocorticoid hypertension, unlike hypertension caused by mineralocorticoid excess, does not require a high salt intake to develop [1, 2]. In this form of hypertension, the renin–angiotensin system is activated presumably as a consequence of a glucocorticoid-induced increase in plasma renin substrate [2]. However, the blood pressure increase of glucocorticoid-treated rats could not be prevented by chronically inhibiting the formation of angiotensin II [3] by using the angiotensin-converting enzyme inhibitor captopril [4]. Furthermore, administration of saralasin, a competitive antagonist of angiotensin II [5], or of captopril [4], did not normalize blood pressure of rats with established glucocorticoid hypertension [2, 6]. In fact, in glucocorticoid hypertensive rats maintained...
on a salt-deficient diet, the blood pressure response to captopril has been found to be smaller than in corresponding normotensive controls [6].

An enhanced vascular smooth muscle sensitivity to noradrenaline is also thought, based on experiments in vitro, to be involved in the pathogenesis of hypertension produced by glucocorticoid excess [7]. This hyper-responsiveness has been attributed to the inhibiting effect of glucocorticoid hormones on the biosynthesis of prostaglandins [8]. In intact glucocorticoid-hypertensive rats, however, no evidence of a hypersensitivity to exogenous noradrenaline has been detected [2].

Recent studies have implicated central adrenaline-containing neurons in the regulation of arterial pressure as well as in the pathogenesis of some types of experimental hypertension [9-12]. It is therefore noteworthy that glucocorticoids are known to induce phenylethanolamine N-methyltransferase (PNMT) activity not only in the adrenals [13], but also at the brain level [14, 15]. Thus, daily injections of dexamethasone to rats have been shown to increase the activity of the adrenaline-forming enzyme in the medulla oblongata within 1 week and in the hypothalamus within 2 weeks [14].

In the present experiments, we evaluated the blood pressure effect of a peripherally and centrally active PNMT inhibitor [16-18], SKF 64139 (7,8-dichloro-1,2,3,4-tetrahydroisoquinoline hydrochloride), administered orally to conscious rats with established glucocorticoid-hypertension. In addition, the participation of angiotensin II and vasopressin in the maintenance of this form of high blood pressure was investigated by using a competitive antagonist of the vasoconstrictor action of each of these two hormones [5, 19].

Material and methods

Male Wistar rats weighing 150-200 g (Charles River Breeding Laboratories) were housed in a temperature and humidity controlled room with automatic lighting in 12 h cycles. All the rats were given a low salt diet (Sodium-deficient rat chow diet; ICN Nutritional Biochemicals, Cleveland, OH, U.S.A.) and were allowed tap water ad libitum throughout the study. Thirty rats were treated with two doses of a methylprednisolone acetate suspension (Depo-Medrol, 40 mg/ml, Upjohn Co., MI, U.S.A.), 20 mg/kg subcutaneously, injected at a 1 week interval. Seventeen other rats received similar injections of two volume-equivalent doses of 5% glucose.

Two weeks after the first injection, under ether anaesthesia, all animals had the right external iliac artery cannulated with a PE-50 catheter and the right femoral vein with a PE-10 catheter. Both catheters contained a heparinized 5% glucose solution. Arterial pressure was then continuously monitored with a Statham transducer and recorded on a Hewlett-Packard recorder (model 7702B). Pulse rate was derived from the blood pressure tracing. Upon awakening, the rats were maintained on a light mesh screen for 60-90 min until blood pressure rose to a steady baseline. At this time, the experiment was started.

The following compounds were used: SKF 64139 (Smith Kline and French Laboratories, PA, U.S.A.) and (Sar1,Ala8)angiotensin II, saralasin (Norwich Pharmacal Co., Norwich, NY, U.S.A.), which were dissolved in 5% glucose to achieve a final concentration of respectively 62.5 mg/ml and 1 mg/ml; in addition, 2 mg of the arginine-vasopressin (AVP) analogue [1-β-mercapto-β-β-cyclopentamethylenepropionic acid, 2-(O-methyl)-tyrosine] arginine-vasopressin [19], was dissolved in a solution made from 10 ml of 0.9% NaCl, 10 mg of bovine serum albumin and 3 μl of acetic acid. This solution was subsequently brought to a pH of 6.4 with NaOH. Rats received by oral gavage either SKF 64139 (50 mg/kg) or a volume-equivalent dose of 5% glucose, 10 μg of saralasin/min, infused by a Harvard pump at a rate of 0.01 ml/min, or 30 μg (0.15 ml) of the AVP antagonist in a bolus intravenous injection.

Blood pressure and heart rate were recorded during 4 h after administration of SKF 64139 in eight normotensive and nine glucocorticoid-hypertensive rats as well as after gavage with 5% glucose in nine normotensive and nine glucocorticoid-hypertensive rats. In all these animals, 0.3 ml of blood was withdrawn at the end of the experiment via the arterial catheter for determination of plasma catecholamines. Plasma noradrenaline and adrenaline levels were assayed, as described elsewhere [20], with a modification of the method of Peuler & Johnson [21]. In our laboratory, the limit of detection is 0.04 ng/ml for adrenaline and noradrenaline. The coefficient of variation for both catecholamines is 5% intra-assay and 8% interassay. In two additional groups of six glucocorticoid-hypertensive rats, the blood pressure and heart rate effect of saralasin or of the AVP-analogue was studied over a 20 min period.

Results

Fig. 1 illustrates the changes in weight occurring in normotensive and glucocorticoid-hypertensive rats...
Mechanisms in glucocorticoid-hypertension

during the 2 weeks before to the day of the experiment. Whereas in the normotensive animals a weight gain of 33.9 ± 2.8 g was observed ($P<0.001$), the glucocorticoid-treated rats failed to grow during the same period and actually decreased their body weight by an average of 23 ± 2.6 g ($P<0.001$).

Table 1 shows the baseline mean blood pressures and heart rates as well as the changes in these observed in normotensive and hypertensive rats during the 4 h observation period after oral gavage with either 5% glucose or SKF 64139. Baseline arterial mean blood pressure was significantly higher ($P<0.001$) in the glucocorticoid-treated rats than in the corresponding normotensive animals, whereas no significant difference in baseline pulse rate was found between the same groups of rats.

The time course of the blood pressure response to 5% glucose or SKF 64139 in these different groups of normotensive and hypertensive rats is depicted in Fig. 2. No blood pressure change was observed during the experiment in the rats given 5% glucose. SKF 64139 induced only a minor, not significant blood pressure decrease in normotensive rats. In contrast, the hypertensive animals progressively lowered their blood pressures from 145 ± 3.2 to 127 ± 4.1 mmHg ($P<0.001$) after SKF 64139 administration. The changes in pulse
TABLE 1. Mean blood pressure, heart rate and plasma catecholamines in normotensive and glucocorticoid-hypertensive rats treated with SKF 64139 or its vehicle

Baseline mean blood pressure (MBP) and heart rate (HR) in (1) normotensive and (2) glucocorticoid-hypertensive rats. The changes in MBP (\(\Delta\) MBP) and HR (\(\Delta\) HR) were measured 4 h after oral administration of 5% glucose or SKF 64139. Plasma noradrenaline and adrenaline levels were determined at the end of each observation period. Mean values ± SEM are shown.

<table>
<thead>
<tr>
<th></th>
<th>Baseline values</th>
<th>(\Delta) MBP (mmHg)</th>
<th>(\Delta) HR (beats/min)</th>
<th>Plasma noradrenaline (ng/ml)</th>
<th>Plasma adrenaline (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Normotensive rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Glucose</td>
<td>119.6 ± 1.4</td>
<td>531.7 ± 8.3</td>
<td>-0.1 ± 1.6</td>
<td>8.3 ± 8.3</td>
<td>0.377 ± 0.036</td>
</tr>
<tr>
<td>SKF 64139</td>
<td>118.9 ± 1.7</td>
<td>515.6 ± 13.9</td>
<td>-3.4 ± 1.6</td>
<td>20.6 ± 11.7</td>
<td>0.493 ± 0.09</td>
</tr>
<tr>
<td>(2) Glucocorticoid-hypertensive rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Glucose</td>
<td>141.4 ± 2.1</td>
<td>551.7 ± 8.2</td>
<td>-2.6 ± 1.3</td>
<td>-7.8 ± 9.7</td>
<td>0.401 ± 0.074</td>
</tr>
<tr>
<td>SKF 64139</td>
<td>144.9 ± 3.2</td>
<td>543.0 ± 9.6</td>
<td>-17.9 ± 3.8*</td>
<td>-45 ± 8.3*†</td>
<td>0.677 ± 0.137</td>
</tr>
</tbody>
</table>

*P < 0.005 (one-way analysis of variance).
†P < 0.001 vs baseline (Student’s t-test).

The rate paralleled the changes in blood pressure (Table 1) and only the glucocorticoid-hypertensive rats treated with SKF 64139 significantly slowed their pulse rate during the observation period (from 543 ± 10 to 488 ± 12 beats/min, P<0.001).

Fig. 3 illustrates the blood pressure effect of saralasin (Fig. 3a) and of the AVP-antagonist (Fig. 3b) administered to two different groups of glucocorticoid-hypertensive rats. Blockade of the renin-angiotensin system resulted in a significant fall in mean blood pressure, which averaged 8.2 ± 1.6 mmHg (P<0.005) at the end of saralasin infusion. On the other hand, the slight blood pressure decrease induced by the AVP-antagonist was not significant. Pulse rate also was not significantly affected by the administration of both inhibitors.

The plasma catecholamine levels determined at the end of the experiment in all 5% glucose- and SKF 64139-treated normotensive and hypertensive rats are shown in Fig. 4 and summarized in Table 1.

Fig. 3. Blood pressure response to (a) saralasin and (b) to an arginine-vasopressin antagonist in glucocorticoid-hypertensive rats. Mean values ± SEM are shown. *P < 0.05; **P < 0.005.
Mechanisms in glucocorticoid-hypertension

No significant difference in plasma noradrenaline and adrenaline levels was observed between normotensive and hypertensive rats. The plasma levels of both catecholamines tended to be higher in the SKF 64139-treated groups of rats than in the corresponding control rats, but the difference did not reach statistical significance.

Discussion

These data confirm that exogenous glucocorticoids produce hypertension when given for a relatively short period to rats maintained on a salt-deficient diet. This diet was chosen to avoid any sodium retention in the hypertensive rats as a possible consequence of a mineralocorticoid effect of high doses of glucocorticoids administered. The weight loss we observed in the glucocorticoid-treated rats might be primarily due to the well-established catabolic effect of adrenocortical hormones. It is also possible that this decrease in body weight is partly the consequence of a negative sodium balance. Indeed, a significantly more pronounced salt depletion has been observed in rats kept on a sodium-deficient diet and given methylprednisolone than in normotensive controls [6]. This glucocorticoid-mediated effect has been attributed to an increased filtered load of sodium in the kidneys [22].

This negative sodium balance [6] together with the enhanced synthesis of plasma renin substrate [2] associated with glucocorticoid treatment is probably responsible for the activation of the renin-angiotensin system, which has been described to accompany the blood pressure rise in the rat [2]. In the present experiment, the participation of the renin-angiotensin system in the maintenance of established glucocorticoid-hypertension was evaluated by using saralasin, a competitive antagonist of angiotensin II [5]. The fall in blood pressure induced by this compound (−8.2 ± 1.6 mmHg) in our hypertensive rats was not impressive, especially when one keeps in mind that the animals were fed with a salt-free diet and that, in addition, glucocorticoids have a natriuretic effect [22]. These findings are close to those reported by other investigators in anaesthetized methylprednisolone-treated rats maintained on a regular sodium intake [2]. It is unlikely that the role of the renin-angiotensin system in sustaining high blood pressure is underestimated in our rats as a result of agonistic properties of saralasin, since the vasoconstrictor action of this competitive antagonist of angiotensin II is known to be particularly weak in a salt-depleted state [23, 24]. Further evidence exists suggesting that other mechanisms than activation of the renin-angiotensin system are involved in the pathogenesis of glucocorticoid-hypertension. Thus chronic angiotensin-converting enzyme inhibition with captopril only partly prevents the development of this type of experimental hypertension [3], and acute blockade of angiotensin II generation with this inhibitor is significantly less effective in lowering blood pressure in rats with glucocorticoid-hypertension than in corresponding normotensive controls [6].

In the present study, we also investigated the role of circulating vasopressin in established glucocorticoid-hypertension. Using a competitive antagonist of the vasoconstrictor action of vasopressin [19], we found that this hormone does not significantly contribute to the maintenance of this form of high blood pressure.

The most interesting features of our experiments appeared to be the different patterns of blood pressure response we observed in normotensive and glucocorticoid-hypertensive rats after oral administration of SKF 64139, a peripherally and
centrally acting PNMT inhibitor [16-18]. The oral dose of SKF 64139 we used is known to decrease by approximately 80% PNMT activity in medulla oblongata of conscious rats over a 60-90 min period [25]. Indeed, whereas in normotensive rats blood pressure was not significantly modified by the investigational compound, hypertensive rats treated with SKF 64139 almost normalized their blood pressures during the 4 h observation period. These findings are particularly interesting since glucocorticoids are known to modulate PNMT activity not only in the adrenal glands [13], but also in the central nervous system [14, 15]. In fact, increased activity of the adrenaline-forming enzyme has been detected in rats after dexamethasone treatment in some brain areas, such as medulla oblongata and hypothalamus [14], which are generally assumed to participate in cardiovascular homoeostasis [26].

Our data clearly indicate that PNMT inhibition at the periphery does not explain the blood pressure response to the inhibitor since plasma adrenaline levels were not suppressed in SKF 64139-treated rats. Conversion of noradrenaline to adrenaline in the rat adrenal is almost completely blocked with an oral dose of 20 mg of SKF 64139/kg [16]. However, adrenaline turnover is very slow in the adrenals [13]. Therefore, even in rats treated with SKF 64139 at 200 mg day⁻¹ kg⁻¹ urinary adrenaline excretion is still not changed after 3 days and 5 days of PNMT inhibition are necessary to reduce adrenaline excretion [27]. Accordingly, the antihypertensive effect of SKF 64139 as a PNMT inhibitor may be due only to central blockade of adrenaline formation. Such a mechanism is compatible with the observation that intracerebroventricular injection of SKF 64139 has recently been shown to attenuate the blood pressure rise in mineralocorticoid-salt and one-clip, one-kidney hypertensive rabbits [28], i.e. in two models of experimental hypertension known to have increased brain stem PNMT activity [9, 11], and to respond to acute parenteral or oral administration of the same inhibitor with a blood pressure decrease [12, 25].

The usefulness of SKF 64139 in delineating the role of adrenaline as a central neurotransmitter remains, however, to be further explored. On the one hand, this potent PNMT inhibitor has been found to be also a weak monoamine oxidase inhibitor in experiments both in vitro [16] and in vivo [29]. On the other hand, the same compound has recently been shown in conscious rats to have some inhibiting properties on β₂-adrenoceptors and neuronal uptake processes [30]. In our experiments, the clear trend of plasma catecholamine levels of both the normotensive and hypertensive rats treated with SKF 64139 to be higher than in corresponding controls is therefore compatible with some degree of α-adrenergic blockade, monoamine oxidase inhibition and/or inhibition of noradrenaline and adrenaline uptake by terminal nerve endings.

It has also to be pointed out that the blood pressure and heart rate response to SKF 64139 is certainly not due to blockade of α-adrenoceptors, since this compound has no affinity at all for these receptors in vitro [16] and does not modify in vivo the chronotropic response to isoprenaline [31]. In addition, our data demonstrate that the pulse rate effect of SKF 64139 is not caused by a decrease in circulating adrenaline levels. Thus the cardiac decelerating effect of this compound may suggest that central adrenaline actively participates in the regulation of the baroreceptor reflex. On the other hand, although the concomitant reduction in blood pressure and heart rate induced in our hypertensive rats by SKF 64139 would be rather unexpected in the face of a significant α-adrenoceptor blockade, such a response would not be surprising as a result of monoamine oxidase inhibition.

Whatever the precise action of SKF 64139 in the central and/or peripheral nervous system, our data strongly support the concept that the sympathetic system is either hyperactive or hypersensitive in the established form of glucocorticoid-hypertension. Interestingly, there was no significant difference in plasma catecholamine levels between our normotensive and hypertensive rats given 5% glucose. Assuming that noradrenaline released in the circulation accurately reflects the peripheral sympathetic activity, our findings would suggest that glucocorticoid-induced blood pressure elevation is mainly the consequence of a vascular smooth muscle hyper-responsiveness to this hormone. In fact, such an explanation has been proposed on the basis of experiments in vitro [7]. In contrast, studies in vivo failed to demonstrate after glucocorticoid treatment any enhancement in vascular reactivity to exogenous noradrenaline [2]. It is, however, possible that plasma noradrenaline levels only poorly reflect the activity of the peripheral and central autonomic nervous system.

Acknowledgment

This work was supported in part by USPHS grant HL-18318.

References

1. Knowlton, A.I. & Loeb, E.N. (1957) Depletion of carcass potassium in rats made hypertensive with desoxycorticosterone acetate (DCA) and with corti-
Mechanisms in glucocorticoid-hypertension