Urinary prostaglandin E$_2$ and kallikrein excretion in glucocorticoid hypertension in rats

MICHIKO HANNA, KAZUOKI KONDO*, HIROMICHI SUZUKI† AND TAKAO SARUTA

Department of Internal Medicine, Keio University School of Medicine, Tokyo, *Department of Clinical Pharmacology, Jichi Medical School, Tochigi, Japan, and †Cleveland Clinic, Ohio, U.S.A.

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Summary

1. Oral administration of dexamethasone (about $2.5 \times 10^{-7}$ mol/day) caused hypertension in rats. The blood pressure rose from $108 \pm 6$ (mean $\pm$ SD) to $156 \pm 17$ mmHg on the seventh day. The urine volume and urinary excretion of sodium were increased. The plasma renin activity and plasma aldosterone were unchanged. However, the urinary excretions of prostaglandin E$_2$ ($U_{PGE2}$V) and kallikrein ($U_{kall}$V) were markedly decreased throughout the experiment.

2. With concurrent administration of captopril, the elevation of blood pressure was partially prevented. In this group of rats, the plasma renin activity was elevated and the reductions in $U_{PGE2}$V and $U_{kall}$V were partially prevented.

3. Based on these results, it is suggested that suppression of the kallikrein-kinin and prostaglandin systems, in addition to involvement of the renin-angiotensin system, is one of the factors contributing to the hypertensive action of dexamethasone.

Key words: glucocorticoid, hypertension, renin-angiotensin system, prostaglandin E$_2$, kallikrein, captopril.

Abbreviation: PG, prostaglandin.

Introduction

Glucocorticoid excess causes hypertension in man [1, 2] and in experimental animals [3–7], but the precise mechanism by which the glucocorticoid induces hypertension remains unclear.

Krakoff et al. [1] reported in 1975 that increased angiotensin formation on the basis of elevation in plasma renin substrate might cause hypertension in Cushing’s syndrome. Subsequently, several studies have been made to elucidate the role of the renin-angiotensin system in this type of hypertension [2, 5, 6, 8], and its partial role has been confirmed. However, in addition to the part played by the renin-angiotensin system, there is a possibility that other mechanisms are involved in the development of this type of hypertension. Since it is known that administration of a large amount of glucocorticoid inhibits phospholipase A$_2$ [9], which is related to the synthesis of arachidonic acid from phospholipids, changes in prostaglandins will be induced by the administration of glucocorticoid. Furthermore, it is conceivable that the kallikrein-kinin system will also be influenced by administration of glucocorticoid, since prostaglandins and the kallikrein-kinin system are intimately related [10].

In the present study, therefore, glucocorticoid hypertension was induced by administering dexamethasone to rats as reported by us previously [11], and the changes in urinary excretion of kallikrein and prostaglandin E$_2$ (PGE$_2$), as well as plasma renin activity and plasma aldosterone, were observed. Furthermore, we examined the effect of captopril, an angiotensin-converting enzyme inhibitor, on these depressor systems, since we recently found that this substance could significantly attenuate the development of dexamethasone-induced hypertension.

Materials and methods

Male Wistar rats weighing 180–200 g were used throughout the experiments. All rats were main-
tained in humidity- and temperature-controlled quarters, each rat being housed in a metabolic cage. All animals were fed on the same stock chow diet (Na, 0.23 g/100 g; K, 0.77 g/100 g).

The rats were divided into four groups. The animals of group 1 (12 rats) were used as controls and were given tap water. The animals of group 2 (16 rats) were given tap water containing 7.6 µmol of dexamethasone/l; each animal took about 2.5 × 10⁻⁷ mol of dexamethasone/day. The dexamethasone was supplied by Nippon Merk Banyu, Japan. The animals of group 3 (16 rats) were given tap water containing 7.6 µmol of dexamethasone/l and 4.3 µmol of captopril/l; each animal took about 2.5 × 10⁻⁷ mol of dexamethasone/day and 2.7 × 10⁻⁸ mol of captopril/day. The captopril was supplied by Sankyo Pharmaceutical Co., Japan. The animals of group 4 (16 rats) were given tap water containing 4.3 µmol of captopril/l; each animal took about 2.7 × 10⁻⁸ mol of captopril/day. All groups of animals were given tap water ad libitum. The fluid intake, urine volume and urinary excretion of prostaglandin E₂ (UₚGₑ₂/ₐ) and kallikrein (Uₖa/l, V) were measured every other day.

The systolic blood pressure was checked every other day and the body weight was measured on the third and seventh experimental days. After measurement of the systolic blood pressure and body weight, half of the animals were decapitated on day 3 of the experiment and the remaining animals on day 7. Blood samples were obtained for assay of the plasma renin activity and the plasma aldosterone concentration.

In the morning of each experimental day, the systolic blood pressure was measured while the animal was conscious, with a tail plethysmographic method [12]. Each rat was restrained in a warmed chamber while at least three consecutive pressure determinations were made. The average of these three values was recorded as the systolic blood pressure for that animal.

The plasma renin activity was measured by the bioassay method of Skinner et al. [13] as reported in our previous study [14]. The plasma aldosterone concentration was determined by radioimmunoassay. The urinary sodium was estimated with a flame photometer (Instrumentation Laboratory, Lexington, MA, U.S.A.).

Urine content of immunoreactive PGE₂ was determined by radioimmunoassay by the method of Dray et al. [15]. Aliquots (3 ml) of the urine sample were acidified to pH 3.0 with citric acid after addition of ³H-PGE₂ tracer, and extracted twice with cyclohexane/ethyl acetate (1:1). The organic phase was removed and evaporated, and the residue was redissolved for chromatography on 0.5 g silicic acid columns equilibrated with benzene/ethyl acetate (3:2). PGE₂ was eluted by the development of the columns with 12 ml of benzene/ethyl acetate/methanol (60:40:2) and this fraction was dried under a stream of nitrogen and redissolved in assay buffer (3 ml) on the day of assay. For the assay procedure, standards of PGE₂ (1-100 µg/ml) or samples (100 µl aliquots assayed in duplicate) were incubated with antibody (Ono Pharmaceutical Co., Japan) for 4 h at 0°C. Bound and free antigens were separated with charcoal/dextran, and the concentration of antibody-bound antigen was determined by scintillation counting of the supernatant. Recovery through the extraction and purification procedure was determined for each sample from tracer ³H-PGE₂ recovery, and was 60%. The coefficient of variation for intra-assay reliability was 8% and inter-assay reliability was 10%.

Urinary kallikrein was measured by the method of Morita et al. [16] using a fluorogenic peptide substrate, prolyl-phenylalanyl-arginine-4-methylcoumaryl-7-amide (Pro-Phe-Arg-MCA). The assay method was as follows. Urine (100 µl) and 20 µl of Pro-Phe-Arg-MCA solution (10 mmol/l) in dimethyl-formamide were added to 210 ml of Tris/HCl buffer (0.1 mol/l), pH 8.0, containing NaCl (0.15 mol/l). The amount of fluorogenic amino-4-methylcoumarine (AMC) liberated was measured by using a Shimazu fluorescence spectrophotometer with excitation at 380 nm and emission at 460 nm. Relative fluorescence was obtained for a solution (10 µmol/l) of AMC in 0.1% dimethyl-sulphoxide. Reliability was examined by reported analysis of pooled urine. The coefficient of variation for analysis of pooled urine was 1.0% (intra-assay reliability) and the inter-assay reliability was 1.8%.

All results were expressed as means ± SD. Comparisons between the groups were made by Student's t-test for unpaired data.

Results

Administration of dexamethasone caused marked increases in blood pressure: the blood pressure rose from 108 ± 6 to 138 ± 16 mmHg on day 3 (P < 0.005) and to 156 ± 17 mmHg on day 7 (P < 0.005) (Fig. 1). With concurrent administration of dexamethasone and captopril, the elevation of blood pressure was significantly (P < 0.005) attenuated. Captopril alone caused slight decreases in blood pressure but these were not significant (Fig. 1).

Administration of dexamethasone resulted in marked decreases in UₚGₑ₂/ₐ and Uₖa/l, V (P < 0.005 and P < 0.005 respectively). The decreases
Mechanism of glucocorticoid hypertension

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FIG. 1. Changes in systolic blood pressure during treatment with dexamethasone (DX) and/or captopril (SQ). Values are mean ± SD. Significance of difference: *P < 0.05 and ***P < 0.005. NS, Not significant. ▲, Control (n = 6); ■, DX (n = 8); ○, DX and SQ (n = 8); △, SQ (n = 8).

in $U_{\text{PGE2V}}$ and $U_{\text{kall.V}}$ were observed before the elevation of blood pressure and continued throughout the experiment. The decreases in $U_{\text{PGE2V}}$ and $U_{\text{kall.V}}$ were partially restored by concurrent administration of captopril with dexamethasone. In rats treated with captopril alone, $U_{\text{PGE2V}}$ was increased slightly ($P < 0.05$), and $U_{\text{kall.V}}$ was not different from that in control rats (Figs. 2 and 3). The plasma renin activity and the plasma aldosterone concentration in dexamethasone-treated rats were not different from those in control rats. Administration of captopril resulted in an increase in plasma renin activity and a decrease in plasma aldosterone concentration in rats with and without dexamethasone treatment (Fig. 4).

Urine volume and urinary sodium excretion were significantly increased both in rats treated with dexamethasone ($P < 0.005$ and $P < 0.005$ respectively) and in those treated with captopril ($P < 0.005$ and $P < 0.005$ respectively). Concurrent administration of dexamethasone and captopril also increased the urine volume and urinary sodium excretion significantly ($P < 0.005$ and $P < 0.005$ respectively). There were no marked differences in urine volume and urinary sodium excretion between rats treated with dexamethasone or captopril and those treated with both dexamethasone and captopril (Fig. 5). Water intake was increased slightly and body weight was decreased in all of these three groups (Fig. 5).

Discussion

We have confirmed our previous finding that dexamethasone treatment induces a significant
blood pressure elevation with increases in urine volume and urinary sodium excretion in rats [11]. We also found out that $U_{\text{PGF}_2\alpha}V$ and $U_{\text{kall}}V$ were markedly reduced and that plasma renin activity and plasma aldosterone concentration were not significantly changed by the administration of dexamethasone. Furthermore, treatment with captopril partially prevented the development of hypertension as well as the decreases in $U_{\text{PGF}_2\alpha}V$ and $U_{\text{kall}}V$ induced by dexamethasone.

The renal prostaglandin system has not yet been extensively examined in glucocorticoid hypertension. It is well known that high doses of glucocorticoid affect the capacity of tissues to produce prostaglandins by inhibiting the release of arachidonic acid from phospholipids [9]. Our results are consistent with this concept. Thus the decreased $U_{\text{PGF}_2\alpha}V$ observed in dexamethasone-treated rats may be due to the direct action of dexamethasone.

There are only a few reports concerning the effect of glucocorticoid on the renal kallikrein-kinin system, and the results are conflicting. As in our study, Bönner et al. [17] reported that high doses of corticosterone caused a marked decrease in $U_{\text{kall}}V$ in rats. Our results and those of Bönner et al. are, however, in contrast to the report of Croxatto et al. [18], who observed that corticosterone treatment slightly enhanced $U_{\text{kall}}V$ in rats. However, the doses of corticosterone employed in their experiments were small. In our preliminary study, we observed that a small dose of dexamethasone (about $1.2 \times 10^{-8}$ mol/day) did not induce any change in blood pressure. It is suggested therefore that high doses of glucocorticoids are needed to produce a reduction in $U_{\text{kall}}V$ as well as an elevation of blood pressure.

Renal kallikrein is released by high arterial pressure, volume expansion, angiotensin II and mineralocorticoids [19]. In the present study, sodium and water excretion were increased, blood
pressure was increased, and plasma renin activity and plasma aldosterone were unchanged in the dexamethasone-treated rats. These factors, therefore, do not explain the decreased $U_{\text{kall}} \cdot V$ induced by dexamethasone. There have been several reports [19-22] which indicate that renal prostaglandins may also participate in the regulation of renal kallikrein excretion. Thus the decreases in $U_{\text{kall}} \cdot V$ observed in this study may be a consequence of suppression of prostaglandins by dexamethasone. Of course, the direct effect of dexamethasone on the renal kallikrein excretion should be considered. However, the possibility that dexamethasone might directly inhibit kallikrein release in the kidney seems unlikely, since Vio et al. [23] recently demonstrated that, in the isolated perfused kidney, dexamethasone in the perfusate did increase the kallikrein release both in the urine and in the venous outflow.

The kallikrein–kinin and prostaglandin systems are considered to be main depressor systems in vivo and are suggested to participate in the control of blood pressure. Elijovich & Krakoff [24] suggested in their recent report that activity of vasodilator systems was decreased in methylprednisolone-hypertensive rats. Therefore, although it is still questionable whether renal excretion of PGE$_2$ and urinary kallikrein may directly indicate the state of circulating or local vascular levels of these vasodilatory systems, our results seem to support the hypothesis that suppression of prostaglandins and the kallikrein–kinin system may play some role in the pathogenesis of dexamethasone-induced hypertension.

Although plasma renin activity was not increased by the administration of dexamethasone, our results do not exclude the involvement of the renin–angiotensin system in this form of hypertension, since sensitivity to angiotensin II could be increased in glucocorticoid hypertension [1]. It was reported that saralasin and/or captopril decreased blood pressure in patients with Cushing’s syndrome [2] or methylprednisolone-treated rats [5, 6, 8]. These results suggest that hypertension in glucocorticoid excess is partially dependent on the renin–angiotensin system. In the present study, concomitant administration of captopril partially prevented both the decreases in $U_{\text{PGE}_2} \cdot V$ and $U_{\text{kall}} \cdot V$ and the development of hypertension in dexamethasone-treated rats. Although the precise mechanisms whereby concomitant administration of captopril can prevent the decreases in $U_{\text{PGE}_2} \cdot V$ and $U_{\text{kall}} \cdot V$ remain obscure, these results do suggest, but do not prove, that the antihypertensive effect of captopril may be mediated by the restoration of depressed renal kallikrein–kinin [25] and prostaglandin systems [26-28], as well as by inhibition of angiotensin II formation. However, other possibilities, such as that the restoration of $U_{\text{PGE}_2} \cdot V$ and $U_{\text{kall}} \cdot V$ was secondary to the restoration of blood pressure, also required consideration. In any case, it is interesting that, in rats treated with both dexamethasone and captopril, a partial prevention of elevation in blood pressure was accompanied by a partial prevention of decreases in $U_{\text{PGE}_2} \cdot V$ and $U_{\text{kall}} \cdot V$.

In conclusion, the present study revealed that $U_{\text{PGE}_2} \cdot V$ and $U_{\text{kall}} \cdot V$ were suppressed in dexamethasone-induced hypertensive rats. It is suggested that suppression of the kallikrein–kinin and prostaglandin systems, in addition to involvement of the renin–angiotensin system, represents one of the factors contributing to the hypertensive action of dexamethasone.

References


