Mechanism of Glucocorticoid-Induced Hypertension

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Despite many previous studies, the mechanism of glucocorticoid-induced hypertension remains unknown. The present review will introduce the results of our studies on this subject in patients with Cushing's syndrome and in experimental animals. Our studies indicate that the following multiple factors are involved in glucocorticoid-induced hypertension in humans and animals: 1) activation of the renin-angiotensin (R-A) system due to an increase in plasma renin substrate (PRS); 2) reduced activity of depressor systems, including the kallikrein-kinin (K-K) system, prostaglandins (PGs), and the endothelium-derived relaxing factor nitric oxide (NO); and 3) increased pressor responses to angiotensin II (Ang II) and norepinephrine. Furthermore, our *in vitro* studies have revealed that the number of Ang II type 1 receptors of vascular smooth muscle cells (VSMCs) is significantly increased by glucocorticoids. It is concluded that the pathogenesis of glucocorticoid-induced hypertension is multi-factorial, involving the various mechanisms described above. (*Hypertens Res* 1996; 19: 1-8)

Key Words: Cushing's syndrome, glucocorticoid, hypertension, pressor response, angiotensin

As exemplified by Cushing's syndrome, an excess of glucocorticoids can cause hypertension (1). However, the pathogenesis of glucocorticoid-induced hypertension remains undetermined, although the involvement of multiple factors has been suggested (2, 3). Over the past several years, our group has performed a series of investigations on this subject in patients with Cushing's syndrome as well in experimental animals (rats and dogs) given glucocorticoids.

Characteristics of Hypertension in Cushing's Syndrome

Hypertension induced by glucocorticoid excess, as seen in Cushing's syndrome, is characterized by: 1) activation of the renin-angiotensin (R-A) system due to an increase in plasma renin substrate (PRS), 2) reduced activity of depressor systems such as the kallikrein-kinin (K-K) system and prostaglandins (PGs), and 3) enhanced pressor responses to norepinephrine and angiotensin II (Ang II). These effects are illustrated in Fig. 1 through 4, which show data obtained from 12 patients with Cushing's syndrome (5 men and 7 women; age, 19-65 years), as compared with data from 6 normal control subjects (3 men and 3 women; mean age, 31 years). Both the patients with Cushing's syndrome and the control subjects were admitted to Keio University Hospital for the study. They were given a regular diet providing 12 g of salt per d. On the morning of the 4th hospital day, blood samples were drawn for the determination of plasma renin activity (PRA),

PRS, plasma renin concentration (PRC), plasma aldosterone concentration (PAC), plasma cortisol (PC), and electrolytes. During the following 24 hours, urine was collected for the measurement of urinary PGE₂ and kallikrein excretion. Pressor responses to Ang II and norepinephrine were examined during infusion of graded concentrations. Blood pressure was monitored continuously during the infusion using an automated blood pressure monitor (Nippon Kohden, Tokyo, Japan). Ang II (Ciba Pharmaceutical Co., Tokyo, Japan) was dissolved in 5% glucose solution and was infused intravenously for 10 minutes at each of the following graded concentrations: 1, 2, 3, 4, 5, and 6 ng/kg per min. Norepinephrine (Eisai Pharmaceutical Co., Tokyo, Japan), also dissolved in 5% glucose solution, was infused similarly at 0.01, 0.03, 0.07, 0.11 and 0.18 μ g/kg per min.

In addition, the effect of an anti-mineralocorticoid drug, spironolactone, was studied in patients with Cushing's syndrome. Unlike patients with primary aldosteronism, the administration of 100 mg per d of spironolactone for 7 days did not reduce blood pressure significantly in patients with Cushing's syndrome (Fig. 5).

Glucocorticoid-Induced Hypertension in Rats

Three mg/kg per day of dexamethasone (DEX), a potent synthetic glucocorticoid, was administered to Wistar male rats (body weight, 175-205 g), with and without salt loading. As shown in Fig. 6, systolic blood pressure rose significantly within 3 days after

Fig. 1. Mean $\pm$ SEM Plasma renin activity, plasma renin concentration, and plasma renin substrate in 12 patients with Cushing's syndrome. The shaded areas are the ranges of the values in normal subjects.

Fig. 2. Mean $\pm$ SEM daily urinary excretion of PGE_2 and kallikrein ($\square$, patients with Cushing's syndrome; $\blacksquare$, normal subjects).

Fig. 3. Mean $\pm$ SEM changes in diastolic pressure in response to graded doses of angiotensin II in patients with Cushing's syndrome ($\bullet$ — $\bullet$) and normal subjects ($\circ$ — $\circ$). * $p < 0.05$; ** $p < 0.01$.

Fig. 4. Mean $\pm$ SEM changes in diastolic pressure in response to graded doses of norepinephrine in patients with Cushing's syndrome ($\bullet$ — $\bullet$) and normal subjects ($\circ$ — $\circ$). * $p < 0.05$; ** $p < 0.01$.

Fig. 5. Antihypertensive effects of spironolactone (100 mg/d) in patients with Cushing's syndrome, primary aldosteronism, and deoxycorticosterone (DOC) excess hypertension.

Fig. 6. Effects of dexamethasone (DEX) with or without 1% NaCl on blood pressure in rats. The bars indicate SEM.

initiating DEX administration, but this effect of DEX on blood pressure was not augmented by the concurrent use of 1% saline solution in place of normal drinking water (4).

The administration of DEX resulted in a marked decrease in the urinary excretion of kallikrein and of PGE₂, while PRA did not change significantly. The pressor response to norepinephrine was enhanced on the 2nd day of DEX administration, at which time blood pressure was still unaltered. The pressor response to norepinephrine was further augmented on the 6th day of DEX treatment.

Glucocorticoid-Induced Hypertension in Dogs

A series of experiments were carried out in dogs to examine the effects of DEX administration on blood pressure and on various hemodynamic and

Fig. 7. Changes in mean arterial pressure (MAP) and heart rate (HR) in high- or low-dose dexamethasone-treated dogs. * $p < 0.05$ vs. the control group. † $p < 0.05$ vs. the low-dose dexamethasone group. Results are mean $\pm$ SEM.

hormonal variables. As shown in Fig. 7, a high dose of DEX, i.e., 0.5 mg/kg per d, induced an abrupt rise in mean arterial pressure (MAP) on day 1 in the conscious dogs, followed by a further gradual elevation of MAP, peaking on day 7 (5). The car-

Fig. 8. Changes in cardiac output (CO), total peripheral resistance (TPR), and renal blood flow (RBF) in high- or low-dose dexamethasone-treated dogs. Results are mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$ vs. the control group. † $p < 0.05$; †† $p < 0.01$ vs. the low-dose dexamethasone group.

diac output decreased and the total peripheral resistance increased significantly, although both urine volume and urinary sodium excretion increased (Fig. 8). Serial observations of hormones revealed that PRA and PAC remained unchanged, PC and AVP were significantly reduced, and plasma levels of atrial natriuretic peptide (ANP) rose significantly (Fig. 9). Urinary excretion of kallikrein, PGE_2 , and 6-keto PGF_1 was significantly reduced in the group given high-dose DEX (Fig. 10).

To confirm that reduced levels of depressor substances such as kallikrein and PGs are involved in the development of glucocorticoid-induced hypertension, depressor effects of exogenous bradykinin and PGI_2 in animals with glucocorticoid-induced hypertension were studied.

As shown in Fig. 11, the administration of bradykinin at $0.5 \mu\text{g/kg}$ per min reduced MAP by 25 ± 5 mmHg in the animals given high-dose DEX, but not in those given low-dose DEX. Likewise, the administration of PGI_2 at $2.0 \mu\text{g/kg}$ per min reduced MAP by 14 ± 3 mmHg in the dogs given high-dose DEX, but not in those given low-dose DEX (6). On the other hand, the bradykinin antagonist D-Arg-(Hyp³, Thi^{5,8}, D-Phe⁷)-bradykinin,

Fig. 9. Changes in plasma renin activity (PRA), plasma aldosterone concentration (PAC), and plasma levels of norepinephrine, atrial natriuretic peptide (ANP), and arginine vasopressin (AVP) in high-dose or low-dose dexamethasone-treated dogs. Results are mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$ vs. the control group.

given as a bolus injection of $200 \mu\text{g/kg}$, elevated MAP significantly in the dogs receiving low-dose DEX, but not in those receiving high-dose DEX.

Thus, our study in conscious dogs showed that a high dose of DEX elevated blood pressure in association with an increase in total peripheral resistance. This latter effect may result from activation of the R-A system as well as the reduced production of depressor substances such as kallikrein and PGs. It should be noted that the rise in blood pressure ensued despite an increase in urinary sodium excretion, which probably occurred due to increased ANP production.

***In Vitro* Studies Using Rat Vascular Smooth Muscle Cells**

In order to elucidate the mechanism of increased pressor responses to vasoactive substances such as Ang II and norepinephrine in glucocorticoid-induced hypertension, we studied the effects of a glucocorticoid on cultured VSMCs. Contraction of VSMCs requires an increase in cytosolic calcium concentration, which in turn is mediated by inositol-phosphate-specific phospholipase C activation. We

therefore studied the effect of DEX on vasoconstrictor-induced inositol triphosphate (IP_3) production in VSMCs.

Fig. 10. Changes in urinary excretion of kallikrein, prostaglandin (PG) E_2 , and 6-keto- $PGF_1\alpha$ before, during, and after cessation of dexamethasone administration. $\square$, Control; ▨ , 0.1 mg/kg per day dexamethasone (low dose); ▩ , 0.5 mg/kg per day dexamethasone (high dose). Values are expressed as mean $\pm$ SEM, $n=6$ in each group. * $p<0.05$; ** $p<0.01$, vs. control.

When VSMCs prepared from the thoracic aorta of Wistar-Kyoto rats were stimulated by 10^{-10} to 10^{-6} M of Ang II, IP_3 was produced in a dose-dependent manner. Pretreatment with 1 μ M of DEX for 48 hours shifted the dose-response curve to the left, as shown in Fig. 12 (7). This effect of DEX required at least 12 hours of incubation to become manifest and was maximum when incubation was prolonged to 24 hours. This enhancement by DEX of Ang II-induced IP_3 production was completely blocked by RU-38486, which is a glucocorticoid-receptor-specific antagonist, indicating that this effect was exerted via glucocorticoid-specific receptors (Fig. 13).

The mechanism by which DEX stimulates Ang II-induced IP_3 production by VSMCs was investigated further by studying the changes in the number and the affinity of Ang II type 1 receptors. The number of Ang II type 1 receptors of VSMCs, measured using the radio-labeled ligand ^{125}I -Ang II, was unchanged during the first several hours of exposure of the cells to DEX. However, the receptor number increased after 12 hours of exposure. The number of the receptors continued to increase subsequently, reaching a level equivalent to $185 \pm 9.8\%$ of control at 48 hours. Figure 14 shows typical binding patterns of the DEX-treated and control cells at 48 hours. The B_{max} value was 27 ± 3 fmol/mg protein in the DEX-treated cells and 15 ± 3 fmol/mg protein in the control cells. The K_d of ^{125}I -Ang II did not change significantly, the K_d of the DEX-treated cells being 1.2 ± 0.3 nmol/l and that of the control cells 1.1 ± 0.2 nmol/l. Furthermore, VSMCs treated with different concentrations of DEX exhibited a dose-dependent increase in the number of Ang II type I receptors (Fig. 15). This effect of DEX on Ang II type I receptors was completely inhibited by RU-38486, a glucocorticoid-receptor-specific antagonist.

In addition to the above studies, the steady-state level of the Ang II type I receptor mRNA was measured by Northern blot analysis. Figure 16 shows

Fig. 11. Effects of infusion of bradykinin (A) or prostaglandin I_2 (A) on mean blood pressure (MAP) in high-dose or low-dose dexamethasone-treated dogs. Results are mean $\pm$ SEM. * $p<0.05$; ** $p<0.01$ vs. the control group. † $p<0.05$ vs. the low-dose group.

the relative changes in Ang II type I receptor mRNA, calculated with the use of a BAS 2000 system. After treatment with DEX for 30 min, Ang II type I receptor mRNA was 2.2 ± 0.3 times higher than control, and it was 7.8 ± 0.7 times higher than control after 24 hours of exposure to DEX (8).

Fig. 12. Dose-response curve of inositol triphosphate (IP_3) production stimulated by angiotensin II in $1\text{-}\mu\text{M}$ dexamethasone-treated ($\bullet$) or untreated ($\circ$) cells. Reactions were stopped 10 seconds after the addition of angiotensin II. Data represent mean $\pm$ SEM of three independent experiments. * $p < 0.05$; ** $p < 0.01$ vs. the value in the control experiment.

Nitric Oxide in Glucocorticoid-Induced Hypertension

Since reduced endothelium-dependent relaxation of vascular smooth muscle has been reported in patients with essential hypertension, decreased nitric

Fig. 13. Bar graph shows the effect of RU-38486 on angiotensin II (Ang II)-stimulated inositol triphosphate (IP_3) production. Vascular smooth muscle cells were incubated with $10\text{ }\mu\text{M}$ RU-38486 with or without $1\text{ }\mu\text{M}$ dexamethasone (DX) for 48 hours. Reactions were stopped 10 seconds after stimulation with 10 nM angiotensin II. Results are mean $\pm$ SEM of three independent experiments. ** $p < 0.01$ vs. the value in the control experiment.

Fig. 14. A typical binding curve (A) and its Scatchard plot (B) for ^{125}I -angiotensin II binding to vascular smooth muscle cells. The cells were exposed to $1\text{ }\mu\text{mol/l}$ dexamethasone ($\bullet$) or ethanol vehicle ($\circ$) for 48 hours. The maximum binding for control was $16.5\text{ fmol/mg protein}$ with a K_d of 1.15 nmol/l , and the maximum binding for dexamethasone-treated cells was $30.5\text{ fmol/mg protein}$ with a K_d of 1.10 nmol/l .

oxide (NO) production has been implicated in several types of hypertension (9, 10).

In our *in vitro* studies using VSMCs, DEX significantly inhibited NO production induced by tumor necrosis factor plus bacterial lipopolysaccharide (11). We therefore determined the urinary excretion of nitrite and nitrate, which are the metabolites of NO, in patients with essential hypertension, in one patient with primary aldosteronism, and in one patient with Cushing's syndrome, both before and af-

ter administration of L-arginine. The control urinary excretion of nitrite and nitrate was significantly less in the patient with Cushing's syndrome than in the other patients. Infusion of L-arginine increased urinary excretion of nitrite and nitrate in all of the subjects studied. However, the production rate of NO, predicted from urinary nitrite and nitrate excretion rates, in the patient with Cushing's syndrome was significantly less than in the other patients (Table 1).

Our studies indicate that induction of hypertension by glucocorticoid excess occurs independently of the level of salt intake. It was further suggested that the following factors are involved in the pathogenesis of glucocorticoid-induced hypertension: 1) suppression of depressor systems, including the K-K system, PGs, and NO; 2) alteration of the R-A system due to increased PRS; and 3) augmented pressor response to humoral substances due to an increased number of receptors such as Ang II type I receptor.

Fig. 15. Bar graph shows the effect of various doses of dexamethasone on the relative increase in the numbers of angiotensin II type 1 (AT_1) receptors. Vascular smooth muscle cells were exposed to dexamethasone for 48 hours. Results are the average of two independent experiments.

Fig. 16. Bar graph shows the time course of changes in the abundance of angiotensin II type 1 (AT_1) receptor messenger RNA (mRNA). The cells were incubated with or without 1 μ mol/l dexamethasone for the indicated time periods. Results represent mean $\pm$ SEM of three independent experiments. * $p < 0.05$ vs. the value in the control experiment.

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Table 1. Time Course of Urinary Excretion (μ mol/mg creatinine per h) of Nitrite and Nitrate in Patients with Secondary Hypertension Given L-Arginine

	Before administration		After administration		
	-2 h	-1 h	1 h	2 h	3 h
Essential hypertension (n=5)	2.1 $\pm$ 0.3	2.2 $\pm$ 0.1	5.7 $\pm$ 1.2	3.0 $\pm$ 1.0	2.4 $\pm$ 0.6
Renovascular hypertension	2.2	2.0	4.9	2.4	2.2
Primary aldosteronism	2.5	2.0	5.2	3.0	2.5
Cushing's syndrome	0.01	0.01	0.07	0.04	0.04

Results in essential hypertension are mean $\pm$ SEM.

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