Glucocorticoids and Atrial Natriuretic Factor Receptors on Vascular Smooth Muscle

Kenichi Yasunari, Masakazu Kohno, Koh-ichi Murakawa,
Koji Yokokawa, and Tadanao Takeda

The effect of glucocorticoids on the atrial natriuretic factor (ANF)–mediated formation of cyclic guanosine monophosphate (cGMP) by intact vascular smooth muscle cells (VSMC) was studied in rats. Cultured VSMC were obtained from the renal arteries of 14-week-old Wistar rats by the explant method. Micromolar concentrations of dexamethasone, given as pretreatment for 48 hours, suppressed the ANF-mediated response. The dexamethasone-induced suppression was detectable at 6 hours and reached a maximum 24 hours after administration in a dose-dependent manner. Inhibitors of protein synthesis blocked this effect of the glucocorticoid. The basal activity of guanylate cyclase in the dexamethasone-treated cells was lower than in the control cells. Other steroids having glucocorticoid action mimicked this suppression of the ANF-mediated response. This suppression was blocked by a glucocorticoid receptor antagonist. The results suggest that glucocorticoids suppress ANF-mediated cGMP formation by VSMC through glucocorticoid type II receptors and the induction of protein synthesis. Suppression of the ANF-mediated response may play a role in glucocorticoid-induced hypertension. (Hypertension 1990;16:581–586)

Interaction of glucocorticoids with the cardiovascular system has been shown to affect cardiovascular function. In the rat, excess glucocorticoid induces a rapid increase in blood pressure.1 Glucocorticoid-induced hypertension is also observed in humans.2 Although it has been reported that glucocorticoids potentiate the response of vascular smooth muscle to the pressor effect of catecholamine,3,4 little is known about the molecular mechanisms by which glucocorticoids affect the blood pressure and the vascular system. A direct effect of glucocorticoid on the vascular receptors is probably involved in glucocorticoid-induced hypertension.5 Consistent with the possibility of direct glucocorticoid effects, classical glucocorticoid receptors that bind dexamethasone have been demonstrated in vascular smooth muscle cells (VSMC).5,7 We recently demonstrated that glucocorticoid enhances prostaglandin E2 and dopamine receptors, which mediate cyclic adenosine monophosphate (cAMP) formation by VSMC. Because prostaglandin E2 and dopamine receptors on VSMC are considered to induce vasodilation, these findings are opposite to glucocorticoid-induced hypertension.

Atrial natriuretic factor (ANF) is a hormone that induces diuresis, natriuresis, and vasodilatation.10 One of the earliest events after binding of ANF to receptors on target cells is an increase in cyclic guanosine monophosphate (cGMP) concentration, indicating that this nucleotide might act as a mediator of such physiological effects as vasodilation.11–13 The membrane form of guanylate cyclase is activated after the binding of ANF to target cells.14,15 More recently it was reported that a membrane form of guanylate cyclase is an ANF receptor.16 In the present study, we examined the effect of glucocorticoids on ANF-induced cGMP formation by the membrane form of guanylate cyclase in VSMC.

Methods

Materials

Type II collagenase, dexamethasone, aldosterone, corticosterone, cortisol, progesterone, β-estradiol, testosterone, 3-isobutyl-1-methylxanthine (IBMX), cycloheximide, and actinomycin D were purchased from Sigma Chemical Co., St. Louis, Mo. Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, trypsin EDTA (Versine), and fetal calf serum (FCS) were purchased from Gibco Laboratories, Grand Island, N.Y. [3H]Guanine and cGMP radioimmunoassay kit were purchased from Amersham Japan Co., Tokyo. Multiwell pipettes and flasks

From the First Department of Internal Medicine, Osaka City University Medical School, Osaka, Japan.

Address for reprints: Kenichi Yasunari, MD, The First Department of Internal Medicine, Osaka City University Medical School, 1-5-7, Asahi-machi, Abeno-ku, Osaka 545, Japan.

Received December 31, 1989; accepted in revised form May 23, 1990.
were purchased from Becton, Dickinson and Co., Oxnard, Calif. Anion exchange chromatography Dowex 50W-X2 was purchased from Bio Rad Laboratories, Richmond, Calif. Rat atrial natriuretic polypeptide (Rat, 1–28) was purchased from the Peptide Institute, Osaka, Japan. RU 38486 was kindly provided by Roussel Uclaf, Paris.

Cell Culture

VSMC were grown from the explants of 14-week-old normotensive Wistar rat renal arteries as previously described.6,9 Animal handling was also done as previously described.13 Cells were identified as VSMC by their morphological and growth characteristics, including growth in multilayers in a typical "hill and valley" pattern, absence of contact inhibition of growth, and the presence of myofibrils on electron microscopic examination. VSMC were grown in DMEM supplemented with 10% FCS. Cells from passage 5 through 10 were used and were subcultured after trypsinization on a weekly basis because cells become confluent in a week. Each plate was replenished twice each week with fresh medium.

Assay for Cyclic Guanosine Monophosphate Formation in Intact Cells

Receptor-mediated cGMP formation was measured by modification of the prelabeling technique of Richelson et al18 based on the column separation method of Fujimoto et al.19 All experiments with VSMC were conducted at least in triplicate by using six-well culture plates. Cells were preincubated with [3H]guanine (2 μCi/ml) at 37°C for 2 hours. Before incubation, cells were rinsed three times with 2 ml DMEM. In most of the experiments, cells were incubated with agonist (ANF) for 10 minutes in 2 ml DMEM in the presence of phosphodiesterase inhibitor IBMX 0.5 mM at 37°C in an atmosphere of 5% CO2 and 95% air. Stimulation of the labeled cells was stopped by adding 1.5 ml of ice-cold trichloroacetic acid (TCA). In some experiments, the culture medium was replaced by DMEM with 10% FCS containing dexamethasone, aldosterone, other steroids, or the vehicle (ethanol) 48 hours before stimulation. TCA extracts were submitted to ion exchange Dowex 50W-X2 column chromatography for the isolation of [3H]cGMP. Thus, each column was washed with 4.4 ml of 0.1N HCl and 1 ml water successively before eluting cGMP with the next 1.5 ml water, which was usually collected directly into the scintillation vial. The recovery of [3H]cGMP through the Dowex column was 85–95%. Radioactivity was determined by liquid scintillation counting. In one experiment, cGMP was measured by an Amersham radioimmunoassay kit. Cells were counted by an electronic cell counter (model Zf, Coulter Electronics, Hialeah, Fla.).

Statistics

Statistical analysis was performed by analysis of variance and Schefte's modified t test. Values of p<0.05 were considered significant.
Effect of Steroid Hormones on Atrial Natriuretic Factor-Mediated Cyclic Guanosine Monophosphate Formation by Vascular Smooth Muscle Cells

Vascular smooth muscle cells were incubated with various steroids for 48 hours. Cells were stimulated by guest on November 1, 2017 http://hyper.ahajournals.org/ Downloaded from

cycloheximide (10 μM) or actinomycin D (5 μg/ml) in the presence or absence of dexamethasone for 48 hours. Both inhibitors completely abolished the response to dexamethasone without altering the ability of the cell to respond to ANF (Table 1).

Effect of Steroid Hormones on Atrial Natriuretic Factor-Mediated Cyclic Guanosine Monophosphate Formation by Vascular Smooth Muscle Cells

Vascular smooth muscle cells were incubated with various steroids for 48 hours. Cells were stimulated by guest on November 1, 2017 http://hyper.ahajournals.org/ Downloaded from

by guest on November 1, 2017 http://hyper.ahajournals.org/ Downloaded from
TABLE 1. Effect of Inhibitors of Protein Synthesis and RNA Synthesis on the Dexamethasone-Mediated Suppression of the Atrial Natriuretic Factor Response

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>[3H]cGMP (dpm/10⁶ cells)</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>3,250±80</td>
<td>1.00</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>2,350±170</td>
<td>1.00</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>2,160±150</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Vascular smooth muscle cells were incubated with 1 µM dexamethasone (DEX) or its vehicle (control) in the presence or absence of the inhibitors for 48 hours, prepared as described in Methods and stimulated with atrial natriuretic factor (ANF) (1 µM). ANF was in the control as well as the DEX-treated cells. Results are expressed as mean±SD from two independent experiments done in triplicate. Unstimulated levels of tritiated cyclic guanosine monophosphate ([3H]cGMP) are 416±16 (control) and 227±20 (DEX-treated cells) dpm/10⁶ cells. *p<0.05. tNot significant compared with control.

Effect of RU 38,486 on Dexamethasone-Induced Suppression of Cyclic Guanosine Monophosphate Formation by Atrial Natriuretic Factor

Treatment for 48 hours with RU 38,486, a specific glucocorticoid receptor antagonist, reversed the dexamethasone-induced suppression of cGMP formation by ANF (Table 3).

Discussion

It has been proposed that ANF receptors be classified as B and C receptors. ANF B receptors are coupled with guanylate cyclase. ANF C receptors are ANF clearance receptors, which are considered to bind to ANF and clear it from the circulation. Most receptors on VSMC seem to be ANF C receptors. It is considered that functions such as vasodilation are caused by guanylate cyclase through ANF B receptors. Therefore, in this study, cGMP was measured to examine the biological ANF receptor response (B receptor response). Our dose–response curve of ANF B receptor is consistent with previous reports.

Our study has shown that the glucocorticoid, dexamethasone, decreases the ANF-mediated cGMP formation by VSMC. Dexamethasone treatment for 48 hours did not affect the cell numbers. The dose–response curve to dexamethasone, the fact that aldosterone had almost no effect on the ANF-mediated cGMP formation, and the fact that this action is reversed by a glucocorticoid receptor antagonist suggest that this action is mediated though glucocorticoid type II receptors. The time course for glucocorticoid action, as well as the fact that inhibitors of RNA and protein synthesis block the effect, strongly support the involvement of a genomic mechanism of action and the requirement for de novo protein synthesis. This behavior is consistent with the general mode of action of steroid hormones. However, like the data on cycloheximide and actinomycin D, these findings should be interpreted with caution because the synthesis of other proteins, including the glucocorticoid receptor itself, could be inhibited at the same time.

There are two types of guanylate cyclase: the membrane form and the soluble form. The membrane form of guanylate cyclase is an ANF receptor. This study shows that ANF receptor response is modified by glucocorticoid. Plasma ANF concentration is also affected by many factors. We reported in hypertensive subjects that plasma ANF is elevated with markedly high blood pressure or left ventricular hypertrophy and that sodium loading increases plasma ANF. Glucocorticoid, which enhances ANF synthesis as well as thyroid hormone, reduces the ANF receptor response.
Glucocorticoids are known to enhance the hormonal stimulation of cAMP accumulation in many cell types including astrocytoma clone D384. We previously reported that glucocorticoid increases prostaglandin E2 and dopamine-mediated cAMP formation in cultured VSMC. Because agonist-induced cAMP formation in VSMC is believed to induce vasodilatation, these findings are contrary to glucocorticoid-induced hyperreactivity of the vascular bed in experimental animals and humans. In the present study, we showed that glucocorticoid decreases ANF-mediated cGMP formation. Because ANF administration is considered to cause vasodilatation through activation of the membrane form of guanylate cyclase (ANF B receptor) in VSMC, these findings are consistent with a glucocorticoid-induced hyperreactivity of the vascular bed and with glucocorticoid-induced hypertension.

The cellular mechanism of glucocorticoid suppression of guanylate cyclase in VSMC remains to be elucidated. Because glucocorticoid increases agonist-induced cAMP formation in VSMC, there is a possibility that this increased cAMP formation increases phosphodiesterase activity, which then increases the breakdown of cGMP. However, suppression occurs in the presence of the phosphodiesterase inhibitor IBMX. Therefore, it is unlikely that increased adenylyl cyclase activity plays some role in this suppression. Glucocorticoids may decrease the number of ANF B receptors. However, even if measurement of ANF B receptor number was possible using a specific ligand, change in ANF B receptor number would be difficult to prove since only a small portion of the ANF receptors in VSMC contain guanylate cyclase (i.e., ANF B receptors). At present, measurement of the ANF receptor number seems to be measurement of the ANF C receptor number. Change in ANF receptor number may not have been helpful in the present study. Even an 80% decrease in ANF C receptor number would not affect the ANF receptor-mediated cGMP formation (ANF B receptor response). Dissociation of the ANF receptor number and the ANF-mediated cGMP formation (i.e., an increased number of ANF C receptors) and blunted guanylate cyclase response has also been reported in spontaneously hypertensive rats. Because the basal level of guanylate cyclase is suppressed by glucocorticoids, a possible mechanism is a direct action of glucocorticoids to suppress the catalytic unit of guanylate cyclase.

We have used a prelabeling technique to measure guanylate cyclase activity. To rule out the possibility that dexamethasone pretreatment affected the prelabeling process and to determine whether the synthesis or catabolism of cGMP was influenced by dexamethasone pretreatment, time course of cGMP formation in response to ANF for the first 2 minutes was also measured by radioimmunoassay. Dexamethasone suppression of ANF receptor response was also observed, and this suppression was observed while the time course curve was linear. Therefore, dexamethasone pretreatment, at least in part, plays a role in the decreased cGMP synthesis.

Because the peripheral vascular regulation of blood pressure is controlled mainly by arteries with only low resistance rather than by large vessels such as renal arteries, which are not considered resistance vessels, we must exercise caution in exploring our results. However, it is unlikely that the ANF-receptor gene in VSMC from renal arteries differs from the ANF receptor gene of other vessels. The advantage of the VSMC culture system used in this study is that the effect of a variety of hormones and drugs on the intact peripheral vascular cells can be individually determined.

In summary, glucocorticoid has been observed to decrease ANF-induced cGMP formation in VSMC. This effect may play a role in the hyperreactive activity of vascular beds and the hypertension induced by glucocorticoids.

Acknowledgment

We thank Machiko Johchi for her technical assistance.

References


Glucocorticoids and atrial natriuretic factor receptors on vascular smooth muscle.
K Yasunari, M Kohno, K Murakawa, K Yokokawa and T Takeda

doi: 10.1161/01.HYP.16.5.581

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/16/5/581