Mineralocorticoids upregulate arterial contraction to epidermal growth factor

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Mineralocorticoids up-regulate arterial contraction to epidermal growth factor. Am J Physiol Regulatory Integrative Comp Physiol 281: R878–R886, 2001.—The present studies test the hypothesis that contraction to EGF is dependent on mineralocorticoids and/or an elevation in systolic blood pressure (SBP). Endothelium-denuded thoracic aortas from sham normotensive, Nω-nitro-L-arginine (L-NNA) hypertensive, Wistar-Kyoto (WKY), and spontaneously hypertensive rats (SHR) were used in isolated tissue-bath experiments. Maximal contraction to epidermal growth factor (EGF; percentage of phenylephrine (PE; 10 μmol/l)-induced contraction) was greater in hypertensive rats compared with sham and WKY rats (17 ± 1 and 12 ± 4%, respectively). Wistar-Furth rats became only mildly hypertensive when given DOCA salt (134 ± 6 mmHg) compared with Wistar rats (176 ± 9 mmHg), but aortas from both strains had a similarly enhanced contraction to EGF (~9 times the maximal contraction of sham aorta). Furthermore, in vitro incubation of aortas from Wistar and Wistar-Furth rats with aldosterone (10 nmol/l) increased EGF-receptor mRNA expression by >50%. These data indicate that arterial contraction to EGF may occur independent of hypertension and be stimulated by mineralocorticoids.

Cardiovascular diseases such as hypertension and atherosclerosis are often associated with an increase in vascular tone or an imbalance favoring the actions of vasoconstrictors. The cellular mechanisms that are altered to result in hypertension are still unknown and the subject of intense investigation. Although it has been suggested that alterations in cellular signaling favoring vasoconstriction are the cause of elevated blood pressure, others contend that the enhanced vascular reactivity observed in hypertension is the result of the rise in blood pressure. The separation of these two variables (increased vascular reactivity and increased blood pressure) is difficult. Epidermal growth factor (EGF), of which the enhanced mitogenic effect on vascular smooth muscle cells from hypertensive rats is well characterized (3, 5, 22), has proven to be a potent vasoconstrictor in DOCA salt-induced hypertension (10). EGF interacts with one of four receptors: ErbB1 (EGFR), ErbB2, ErbB3, or ErbB4. Of these receptors, the primary receptor for EGF is EGFR. EGF-induced contraction was reduced by tyrosine kinase inhibitors, both general and specific to the tyrosine kinase intrinsic to the EGF receptor, and appears to be enabled by an increased density of EGF receptors in the vascular smooth muscle of the DOCA-salt hypertensive rat. It was observed that the contraction to EGF appeared only after the systolic blood pressure (SBP) of the DOCA-salt rats was significantly elevated (10). These findings suggested that vascular changes necessary to enable the contractile response to EGF may be dependent on an increase in SBP.

Separating out the effects of elevated blood pressure and changes in vascular reactivity can be difficult, and thus one model we have chosen to test the above hypothesis is a rat strain that is resistant to mineralocorticoid-induced hypertension. The Wistar-Furth rat, a substrain of the Wistar rat, is relatively resistant to the hypertensive effects of excess mineralocorticoids (21). Others have demonstrated that contraction to norepinephrine and a membrane-depolarizing concentration of potassium chloride is enhanced in arteries from aldosterone-treated Wistar rats and unchanged in aldosterone-treated Wistar-Furth rats (1), suggesting that vascular signaling is altered in response to a sustained elevation in blood pressure. Our findings in the present studies have led to the focus of this paper investigating the possibility that mineralocorticoids themselves may function at the level of the vasculature to increase EGF-receptor expression and thereby enable EGF-induced contraction.

A secondary aspect of the present study was to determine whether an increase in blood pressure is necessary for increased contraction to EGF. It must be noted that a finding of a vascular alteration that proceeds rather than precedes hypertension does not mean that the vascular alteration is not important to the hypertension. Although it is clear that such vascu-
lunar change is not responsible for the initiation of the hypertension, changes can play a role in the maintenance of hypertension. For example, hyperreactivity to serotonin (5-hydroxytryptamine (5-HT)) occurs coincident with or just after an increase in blood pressure, and blockade of appropriate 5-HT receptors cannot decrease blood pressure in weeks 1 and 2 of DOCA-salt hypertension. This, however, changes in weeks 3 and 4 of DOCA-salt hypertension (29).

**Goal.** Thus the aim of this project was to examine contraction of isolated rat aorta to EGF in different forms of hypertension [the DOCA-salt, Nω-nitro-L-arginine (L-NNA) (6), and spontaneously hypertensive rat (SHR) models of hypertension]. In addition, we examined contraction to EGF in isolated aortas from Wistar DOCA-salt hypertensive rats and in aortas from Wistar-Furth rats on DOCA-salt. In complementary in vitro experiments, aortas from Wistar and Wistar-Furth rats were exposed to aldosterone to determine whether mineralocorticoids, independent of blood pressure, could change EGFR mRNA expression. The effect of high salt and DOCA therapy alone on contraction to EGF was also examined. Throughout these studies, changes in contractile responses of EGF were compared with those of 5-HT, to which the vascular effects in hypertensive vessels have been well characterized (26). Collectively, these experiments investigated the dependence of contraction to EGF on mineralocorticoids and the association of an increased response to EGF with elevated blood pressure.

**MATERIALS AND METHODS**

**Animals.** All animal procedures were followed in accordance with institutional guidelines established by Michigan State University. When the rats arrived at our facility, they were housed in clear plastic boxes with wood-chip bedding and allowed ad libitum access to standard rat chow (Teklab) and tap water.

**DOCA-salt hypertension.** Sprague-Dawley rats (225–250 g) were purchased from Charles River (Portage, MI); Wistar and Wistar-Furth rats (200–225 g; age matched to Sprague-Dawley rats) were purchased from Harlan Laboratories (Indianapolis, IN). Under methoxyflurane (Metophane, Mallinckrodt Veterinary, Mundelin, IL) anesthesia, the area to be incised was shaved free of fur. The animals underwent uninephrectomy (flank incision, left side), and a Silastic (Dow Corning, Midland, MI) implant impregnated with DOCA (200 mg/kg) was implanted subcutaneously in the subscapular region. Sham rats were uninephrectomized but did not receive the DOCA implant.

After surgery, Sprague-Dawley, Wistar, and Wistar-Furth DOCA-treated rats received water supplemented with 1.0% NaCl and 0.2% KCl; Sprague-Dawley, Wistar, and Wistar-Furth sham animals received normal tap water. To examine the influence of either DOCA alone or high salt alone, some Sprague-Dawley rats received the DOCA implant and normal tap water, whereas other rats received the high-salt solution but not the DOCA implant. All animals were fed standard rat chow and had ad libitum access to both food and water. After 28 days, SBPs were measured using a standard tail-cuff method.

**L-NNA hypertension.** Sprague-Dawley rats (250–300 g, Harlan Laboratories, Indianapolis, IN) received either normal tap water (sham) or tap water supplemented with L-NNA (0.5 g/l; Sigma Chemical, St. Louis, MO) for 14 days. The rats had ad libitum access to normal rat chow. On day 14, SBPs were measured using a tail-cuff method.

**Wistar-Kyoto rats and SHR.** Wistar-Kyoto (WKY) rats and SHR (12 wk old) were obtained from Charles River Laboratories. The rats had ad libitum access to normal rat chow. SBPs were measured using a tail-cuff method.

**Isolated tissue bath protocol.** Rats were euthanized (80 mg/kg ip pentobarbital sodium), and the thoracic aortas were removed. Arteries were dissected into helical strips (0.25 × 1 cm), and the endothelial cell layer was removed by rubbing the luminal side of the vessel with a moistened cotton swab. Tissues were placed in muscle baths filled with warmed (37°C), aerated (95%O2/5%CO2) physiological salt solution containing (mmol/l): 130 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4·7H2O, 1.6 CaCl2·2H2O, 14.9 NaHCO3, 5.5 dextrose, and 0.03 CaNa2EDTA. One end of the preparation was attached to a glass rod, the other attached to a force transducer (PT03, Grass Instruments, Quincy, MA), and the strip was placed under optimum resting tension (1,500 mg for all tissues) and allowed to equilibrate for 1 h. Changes in isometric force were recorded on a Grass polygraph (Grass Instruments, Quincy, MA). After a 1-h equilibration, arteries were challenged with a maximal concentration of the α1-adrenergic receptor agonist phenylephrine (PE; 10 μmol/l). This response is listed in milligrams in the figure legend of each figure for each group. Tissues were washed, and the status of the endothelium was examined by observing arterial relaxation to the endothelium-dependent agonist acetylcholine (1 μmol/l) in tissues contracted by a half-maximal concentration of PE (~10 nmol/l). Only strips that demonstrated minimal relaxation (~15%) of the PE-induced contraction were used in this study. EGF (10 μmol/l to 300 nmol/l) was incubated with the tissue for ~10 min to allow the contraction to plateau before the addition of the next concentration. Cumulative concentration response curves to 5-HT (1 nmol/l to 300 μmol/l) were conducted in separate aortic strips. In all experiments, the EGF vehicle (10 μmol/l acetic acid + 1 mg/ml bovine serum albumin) was without effect on arterial tone.

**In vitro incubation of arteries with aldosterone.** To assess whether the observed effects were a direct effect of aldosterone, aortas were removed from Wistar and Wistar Furth rats, cleaned, and incubated for 8 h under tissue culture conditions in PBS or PBS + aldosterone (10−8 mol/l). RNA was extracted, and RT-PCR was carried out for EGFR (described in RT-PCR protocol).

**RT-PCR protocol.** RNA was extracted from aortas using a preparative kit (Qiagen), and 1 μg of RNA was used for first strand cDNA synthesis using oligo(dT) as a primer. Occasional RNA samples were subjected to the PCR procedure without prior reverse transcription to control for the presence of contaminating genomic DNA in the sample. PCR amplifications were carried out on a portion of the cDNA produced. Reactions were performed using a PE Applied Biosystems GeneAmp thermal cycler and Thermus aquaticus (Tag) DNA polymerase. The reaction contained 5 pmol/l of each oligonucleotide primer, 200 μmol/l dNTP, 0.2 units Taq, 1.5 μmol/l magnesium chloride, and 1 μCi of [32P]dCTP in the manufacturer's buffer. Optimum annealing temperature, cycle number, and template dilution factor were determined for each amplicon before experimentation. The cDNA (487-bp product) was resolved on an 8% polyacrylamide gel, and the amount of DNA present was identified by phosphorimaging analysis (Bio-Rad, Hercules, CA) and quantified using Multi-Analyst software. The results were normalized to the expres-
Table 1. *SBP and EC*$_{50}$ *values for EGF and maximal contractile responses to EGF*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SBP, mmHg</th>
<th>–log EC$_{50}$, mol/l</th>
<th>Maximal EGF Response, %PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>130 ± 6(5)</td>
<td>7.86 ± 0.59</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>t-L-NNa</td>
<td>213 ± 9(5)*</td>
<td>9.37 ± 0.34</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>WKY</td>
<td>117 ± 1(5)</td>
<td>9.30 ± 0.38</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>SHR</td>
<td>153 ± 2(5)*</td>
<td>9.86 ± 0.30</td>
<td>53 ± 8†</td>
</tr>
<tr>
<td>Wistar sham</td>
<td>120 ± 4(8)</td>
<td>NA</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Wistar DOCA salt</td>
<td>176 ± 9(8)*</td>
<td>9.62 ± 0.14</td>
<td>35 ± 3†</td>
</tr>
<tr>
<td>Wistar-Furth sham</td>
<td>112 ± 7(5)</td>
<td>NA</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Wistar-Furth DOCA salt</td>
<td>134 ± 6(9)</td>
<td>9.57 ± 0.33</td>
<td>34 ± 9†</td>
</tr>
<tr>
<td>Sprague-Dawley sham</td>
<td>113 ± 1(4)</td>
<td>NA</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Sprague-Dawley salt-treated</td>
<td>151 ± 9(5)*</td>
<td>9.61 ± 0.10</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>Sprague-Dawley DOCA alone</td>
<td>156 ± 11(4)*</td>
<td>9.87 ± 0.71</td>
<td>16 ± 9†</td>
</tr>
<tr>
<td>Sprague-Dawley DOCA salt</td>
<td>207 ± 25(4)*</td>
<td>9.43 ± 0.41</td>
<td>67 ± 22†</td>
</tr>
</tbody>
</table>

Values represent means ± SE for the number of animals in parentheses. Epidermal growth factor (EGF) response was calculated using a nonlinear regression analysis: effect = maximum response/1 + (EC$_{50}$/agonist concentration). *Statistical difference (P < 0.05) between systolic blood pressure (SBP) of sham and corresponding hypertensive rats. †Statistical difference (P < 0.05) between maximal response to EGF between sham and hypertensive rats. NA, not calculable; L-NNa, N^-nitro-L-arginine; WKY, Wistar-Kyoto.

**RESULTS**

Table 1 compiles the SBPs of the experimental groups and pharmacological parameters for EGF-induced contraction. We have also included blood pressure data on the graphs depicting EGF-induced contraction. Finally, Table 2 compiles pharmacological parameters for 5-HT, an agonist used in parallel for comparative purposes.

**Contraction to EGF in aortas from sham and l-NNa hypertensive rats.** The l-NNa-induced model of experimental hypertension was used to raise SBP by inhibiting the enzyme nitric oxide (NO) synthase (NOS) and thus the production of the vasodilator NO. The SBP of the l-NNa-treated rats (213 ± 9 mmHg) was significantly elevated compared with that of sham rats (130 ± 6 mmHg; Table 1, Fig. 1A). Aortic strips from the l-NNa hypertensive rats displayed an increased contraction to EGF compared with aortas from sham rats with respect to both potency and efficacy (Fig. 1A). The contractile agonist 5-HT contracted aortic strips from both sham and l-NNa rats; however, the maximum contraction to 5-HT was only slightly greater in strips from l-NNa rats (117.7 ± 5.3%) than in strips from sham rats (94.8 ± 3.0%; Table 2).

**Contraction to EGF in aortas from WKY rats and SHR.** We next determined whether EGF would cause contraction in aortas from genetically hypertensive rats. SHR and WKY rats were purchased at 12 wk of age. At this time point, the SBPs of the SHR rats (153 ± 2 mmHg) were significantly higher than those of the WKY rats (117 ± 1 mmHg; Table 1, Fig. 1B). As observed with the aortas of l-NNa hypertensive rats, a profound contraction to EGF was observed in aortas from SHR rats, whereas minimal contraction was demonstrated in aortas from WKY rats. The EC$_{50}$ values of

Table 2. EC$_{50}$ values and maximal contractile responses to 5-HT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>–log EC$_{50}$, mol/l</th>
<th>Maximal 5-HT Response, %PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>5.95 ± 0.09(5)</td>
<td>94.8 ± 3.0</td>
</tr>
<tr>
<td>t-L-NNa</td>
<td>6.12 ± 0.05(5)</td>
<td>111.7 ± 5.3</td>
</tr>
<tr>
<td>WKY</td>
<td>5.94 ± 0.06(5)</td>
<td>99.7 ± 3.0</td>
</tr>
<tr>
<td>SHR</td>
<td>6.27 ± 0.03(5)</td>
<td>171.0 ± 2.9</td>
</tr>
<tr>
<td>Wistar sham</td>
<td>6.12 ± 0.05(8)</td>
<td>112.0 ± 5.0</td>
</tr>
<tr>
<td>Wistar DOCA salt</td>
<td>6.13 ± 0.03(8)</td>
<td>145.0 ± 12.0</td>
</tr>
<tr>
<td>Wistar-Furth sham</td>
<td>5.76 ± 0.04(9)</td>
<td>102.0 ± 4.0</td>
</tr>
<tr>
<td>Wistar-Furth DOCA salt</td>
<td>6.02 ± 0.02(9)</td>
<td>133.0 ± 9.0</td>
</tr>
<tr>
<td>Sprague-Dawley sham</td>
<td>6.14 ± 0.11(5)</td>
<td>93.9 ± 5.0</td>
</tr>
<tr>
<td>Sprague-Dawley salt-treated</td>
<td>6.29 ± 0.12(5)</td>
<td>133.1 ± 12.8</td>
</tr>
<tr>
<td>Sprague-Dawley DOCA alone</td>
<td>6.25 ± 0.04(4)</td>
<td>108.5 ± 7.0</td>
</tr>
<tr>
<td>Sprague-Dawley DOCA salt</td>
<td>6.08 ± 0.08(5)</td>
<td>116.0 ± 17.9</td>
</tr>
</tbody>
</table>

Values represent means ± SE for the number of animals in parentheses. 5-hydroxytryptamine (5-HT) response is expressed as a %PE-induced contraction (10 μmol/L). 5-HT EC$_{50}$ values were calculated using a nonlinear regression analysis: effect = maximum response/1 + (EC$_{50}$/agonist concentration). *Statistical difference (P < 0.05) between treated animals and their appropriate sham.
from L-NNA and SHR are consistent with this idea. Thus Wistar and Wistar-Furth sham and DOCA-salt rats were used because Wistar rats are sensitive to the hypertensive effects of DOCA salt, whereas Wistar-Furth rats are reported to not become hypertensive with the same treatment (1, 21). If an elevated blood pressure was necessary to observe an enhanced arterial response to EGF, then aortas from Wistar-Furth rats that do not get hypertensive when given DOCA salt should not respond to EGF. Table 3 reports the SBPs of these four groups of rats and the result of a statistical comparison of these blood pressures. Wistar DOCA-salt rats (176 ± 9 mmHg) had significantly higher blood pressures than Wistar shams (120 ± 4 mmHg; Table 3). The maximal contraction to EGF in aortas from Wistar DOCA-salt rats (35 ± 3% maximal PE-induced contraction) was nine times greater than the maximal contraction to EGF in aortas from Wistar sham rats (4 ± 3%; Fig. 2A). Interestingly, the Wistar-Furth DOCA-salt group, whose blood pressures (134 ± 6 mmHg) were only slightly but significantly elevated compared with the Wistar-Furth sham group (112 ± 3 mmHg) but statistically similar to the Wistar sham group (Table 3), achieved the same maximal contraction to EGF as observed in the Wistar DOCA-salt group (34 ± 9% maximal PE-induced contraction or 9 times greater than sham response; Fig. 2B). The concentration of EGF that caused a half-maximal contraction in aortas from Wistar and Wistar-Furth DOCA-salt rats was similar (9.62 ± 0.14 and 9.57 ± 0.33, respectively; Table 1). In addition, although maximal contraction to 5-HT was significantly greater in arteries from Wistar DOCA-salt rats and Wistar-Furth DOCA-salt rats compared with the corresponding sham rats, the absolute increase in contraction to 5-HT (Table 2) was not similar to the absolute increases in contraction observed with EGF.

Because of the slightly elevated blood pressures present in the Wistar-Furth group, we could not discern whether the small increase in blood pressure was responsible for enabling the increased contraction to EGF observed in arteries from Wistar-Furth DOCA-salt rats. Thus we separated out rats in which the blood pressure of the Wistar-Furth sham and Wistar-Furth DOCA-salt rats were matched for blood pressure (Table 3). Systolic blood pressures of Wistar and Wistar-Furth sham and DOCA-salt groups

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar sham</td>
<td>120 ± 4(8)</td>
</tr>
<tr>
<td>Wistar DOCA salt</td>
<td>175 ± 9(8)</td>
</tr>
<tr>
<td>Wistar Furth sham</td>
<td>112 ± 3(9)</td>
</tr>
<tr>
<td>Wistar-Furth DOCA salt</td>
<td>134 ± 6(9)</td>
</tr>
</tbody>
</table>

Values represent means ± SE for the number of animals in parentheses. Newman-Keuls multiple-comparison test: Wistar-Furth sham vs. Wistar DOCA salt, P < 0.05; Wistar-Furth sham vs. Wistar-Furth DOCA salt, P < 0.05; Wistar sham vs. Wistar-Furth DOCA, P < 0.05; Wistar-Furth DOCA salt vs. Wistar DOCA-salt, P < 0.05.
and compared the response of the arteries of those animals to EGF. We studied eight pairs of Wistar sham and DOCA rats and nine pairs of Wistar-Furth sham and DOCA rats. All Wistar DOCA rats had elevated blood pressures compared with Wistar sham rats. In the Wistar-Furth strain, five pairs of shams and rats given DOCA salt had similar, nonelevated blood pressures (115 ± 6 mmHg). In four pairs, the blood pressure of the Wistar-Furth given DOCA salt was raised anywhere from 15 to 60 mmHg above the Wistar-Furth sham. One such set of data is depicted in Fig. 3. It is clear that DOCA-salt treatment, in the absence of elevated SBP, can increase responsiveness to EGF in aortas of the Wistar-Furth rat. Thus these data suggest that mineralocorticoids may directly influence EGF signaling and/or function; this idea should be addressed directly in later experiments.

Contraction to EGF in aortas from sham, salt-alone- and DOCA-alone-treated rats. To determine the influence of DOCA alone and high salt alone on contraction to EGF, Sprague-Dawley rats were placed on either DOCA (200 mg/kg DOCA, normal tap water) or high salt (no DOCA; 1.0% NaCl + 0.2% KCl) for 4 wk. Rats placed on high salt alone (151 ± 9 mmHg) had significantly higher SBPs than those of sham rats (113 ± 1 mmHg Table 1, Fig. 4A). Interestingly, rats on high sal...
salt, although they were hypertensive, did not demonstrate a contraction to EGF (9 ± 5%) that was of the same magnitude as that observed in aortas from DOCA-salt rats (67 ± 22%; Fig. 4A). To the contrary, the maximum response to 5-HT was significantly greater in strips from salt-alone-treated rats (133.1 ± 12.6%) than those observed in sham strips (93.0 ± 5.0%). DOCA alone also significantly raised the SBPs of DOCA rats (156 ± 11 mmHg) compared with sham rats (113 ± 1 mmHg). However, as seen in Fig. 4B, the contractile response to EGF was modestly increased in aortas from DOCA-alone-treated rats compared with sham rat aortas. DOCA alone did not affect contraction to 5-HT (Table 2).

Correlation of blood pressure with maximum arterial contraction to EGF. To determine whether enhanced contraction to EGF was associated with increasing SBP, a linear regression correlation was conducted using all of the sham and hypertensive rats in this study. There was a positive correlation (r = 0.733) between the maximal contraction to EGF and SBP (Fig. 5). However, it is clear that this correlation is not perfect, and there are groups that clearly deviate from a linear association with SBP, namely Wistar-Furth DOCA-salt, SHR, L-NNA, and even DOCA-salt groups. These data provide evidence that high blood pressure is associated with an enhanced contraction to EGF and may be one cause of an enhanced response to EGF, but the lack of a perfect correlation suggests that other factors, acting either independently or in concert with changes in blood pressure, ultimately determine arterial response to EGF.

Effect of aldosterone on EGF-receptor mRNA expression in aortas from Wistar and Wistar-Furth rats. The above experiments using Wistar-Furth rats suggest that mineralocorticoids may be an independent factor for increasing EGF-receptor expression. To examine this idea, we incubated aortas from the Wistar rats, an animal sensitive to the hypertensive effects of mineralocorticoids, and the Wistar-Furth rat, an animal relatively insensitive to the hypertensive effects of mineralocorticoids, with the mineralocorticoid aldosterone (10 nmol/l) for 8 h. RT-PCR was performed, and the results of these experiments were depicted in Fig. 6. EGF-receptor mRNA was detected in aortas from both Wistar and Wistar-Furth rats, and aldosterone significantly increased the density of EGF mRNA in aortas from both strains of rats (P < 0.05). These data confirm, as suggested above, that mineralocorticoids can directly increase EGF-receptor mRNA.

DISCUSSION

Since the discovery of EGF, several EGF-related ligands have been identified including transforming growth factor-α (TGF-α), heparin-binding EGF-like growth factor (Hb-EGF), betacellulin, amphiregulin, and epiregulin. Four different EGF-receptor subtypes have been discovered including EGF (ErbB1) receptor, ErbB2, ErbB3, and ErbB4 (28). Elucidating potential vascular effects, such as contraction, of EGF is crucial because several of these EGF-related growth factors are produced within the vascular wall. For instance, messenger RNA for Hb-EGF is not only found in arteries, but can be concentration and time dependently increased by hydrogen peroxide-generating compounds.

Fig. 5. Correlation of systolic blood pressure and maximum contraction to EGF [reported as %PE (10 μM) contraction] for aortas for all groups of rats.

Fig. 6. A: representative phosphorimage of EGF receptor (EGFR) and cyclophilin mRNA density in Wistar and Wistar-Furth aortas treated with vehicle or aldosterone (Aldo; 10 nmol/l). *RT = no RT control. B: normalized results from RT-PCR for EGFR expression in aortas incubated with vehicle or Aldo. Bars represent means ± SE for triplicates performed in aortas from 8 Wistar and 8 Wistar-Furth rats. *Significant difference (P < 0.05) in relative EGFR density between vehicle- and Aldo-incubated aortas.
As expected, contraction to Furth rats are sensitive to the hypertensive effects of EGF was addressed. Wistar and Wistar-Furth rats present experiments was to investigate the dependence of vascular contraction to EGF on blood pressure. Thus one purpose of the present experiments was to investigate the dependence of vascular contraction to EGF on blood pressure.

Contraction to EGF was profoundly increased in aortas from not only experimentally hypertensive rats (DOCA-salt and L-NNA models of hypertension), but also in vessels from genetically hypertensive rats (SHR). In 1981, Mangiarua et al. (19) reported that by day 10, Wistar rats on DOCA-salt therapy had significantly higher blood pressures and increased DNA synthesis in arteries from DOCA-salt rats compared with shams. However, by day 30 of therapy, DNA synthesis was similar between sham and DOCA salt rats, suggesting that increase synthesis occurred primarily within the first days of treatment. Together, these studies suggest that during the initial development of hypertension, significant cellular changes occur within the vascular wall and may promote or allow enhanced EGF-receptor signaling and vascular contraction.

EGF in Wistar-Furth rats. With the observation that contraction to EGF occurs in arteries from multiple forms of experimentally hypertensive rats, the idea that contraction to EGF is dependent on an elevation in blood pressure or that elevated blood pressure could be a stimulus for the appearance of a contraction to EGF was addressed. Wistar and Wistar-Furth rats were placed on DOCA-salt therapy. Previous studies have determined that Wistar rats but not Wistar-Furth rats are sensitive to the hypertensive effects of mineralocorticoids (1, 21). As expected, contraction to EGF occurred in aortas from Wistar DOCA-salt rats but not from Wistar shams. Surprisingly, EGF stimulated a virtually identical contractile response in aortas from Wistar-Furth DOCA-salt rats as that observed from Wistar DOCA-salt rats. This was, however, in the presence of a small increase in SBP. In contrast, 5-HT stimulated contraction in vessels from all sets of rats, although the maximal contraction obtained was greater in aortic strips from Wistar and Wistar-Furth DOCA-salt rats. It must be mentioned that the Wistar and Wistar-Furth rats received the same dose of DOCA (200 mg/kg) and were treated for the exact same time period, and mineralocorticoids are absorbed similarly between Wistar and Wistar-Furth rats (21). In addition, water intake was also monitored, and both strains of rats drank equal amounts of water when corrected for body size (data not shown). Thus the difference in blood pressure between the two groups must be due to differences in mineralocorticoid sensitivity and not due to differences in DOCA and/or saltwater intake. In a different study, Ullian et al. (27) found that although contraction to angiotensin II and phenylephrine was potentiated in aortic rings from Wistar rats on DOCA-salt therapy, the responses were not potentiated in rings from Wistar-Furth DOCA-salt rats. Similar findings were also observed for norepinephrine and potassium chloride in aldosterone-treated Wistar rats (enhanced) and Wistar-Furth rats (unchanged) (1).

To separate out the potential independent effects of mineralocorticoids and blood pressure on EGF-induced contraction, we went back to the Wistar-Furth DOCA experiments and could separate out animals (about one-half) that did not become hypertensive with DOCA served from Wistar-Furth animals that did increase in blood pressure in response to DOCA salt. We matched pairs of Wistar-Furth DOCA-salt and sham animals that had similar, nonelevated blood pressures and in Fig. 3 depicted the response of the aortas of one pair of animals to EGF. The finding that the EGF-induced contraction was similarly enhanced in nonhypertensive Wistar-Furth DOCA-salt rats and hypertensive Wistar DOCA-salt rats suggests that treatment with DOCA salt must be stimulating the tissue’s responsiveness to EGF. One potential explanation is that DOCA itself upregulates the response to EGF, and thus we tested the ability of DOCA or salt alone to increase arterial contractility to EGF. In neither group of animals, both of which had similarly elevated systolic blood pressures, was the magnitude of increase in contractility to EGF observed, as was seen in DOCA-salt hypertensive rats. The lack of contraction to EGF caused by DOCA is at odds with the data from the Wistar-Furth experiments and the in vitro experiments in which aldosterone directly increased EGFR mRNA. This is difficult to explain, but one speculation may be that the Sprague-Dawley rat is less sensitive to the effects of mineralocorticoids than Wistar-derived strains of rats such that DOCA alone was not able to enable contraction to EGF. Because we do not know the promoter region and potential mineralocorticoid response elements of the EGF receptor in the Wistar-
Furth rat compared with the Sprague-Dawley rat, this remains a speculation.

Together with our findings, these data suggested that DOCA or mineralocorticoids may specifically modulate arterial response to EGF, independent of elevated blood pressure, as the contraction to EGF but not other vasoconstrictors was enhanced in vessels from both Wistar and Wistar-Furth DOCA-salt rats. This was confirmed with findings from in vitro experiments in which aldosterone caused a statistically significant increase in EGF-receptor mRNA density in aortas from both Wistar and Wistar-Furth rats. We have yet to confirm that EGF-receptor protein is increased, and thus it remains unknown as to whether an increase in EGF-receptor mRNA results in an increase in EGF-receptor protein. Nonetheless, mineralocorticoids must be considered as factors that have the potential to upregulate an important protein such as the EGF receptor.

**Association of blood pressure with response to EGF.** It is fair to suggest that mineralocorticoids may have the potential to act independently of changes in blood pressure to enhance reactivity to EGF, but it is possible and likely that, in concert, elevations in blood pressure and mineralocorticoids have complex and interwoven effects on arterial function. A positive correlation was demonstrated between maximal contraction to EGF and SBP (Fig. 5). However, these variables are only partially associated, because contraction to EGF would have been observed in aortas from salt-treated (hypertensive) rats and not in aortas from some Wistar-Furth DOCA-salt rats (not hypertensive). Were the response to EGF purely blood pressure dependent, then none of the aortas from these animals should have responded to EGF. By comparison, contraction to 5-HT was enhanced in vessels from salt-treated rats but not in vessels from rats on DOCA therapy alone, and both of these groups had similar blood pressures. These findings suggest that the pathway that regulates contraction to EGF may be more sensitive to the effects of DOCA or mineralocorticoids, whereas the pathway that regulates contraction to 5-HT are more tightly regulated by changes in blood pressure. These findings also refute the idea that contraction to EGF is solely dependent on a sustained elevation in blood pressure and further suggest that the contractile response to EGF may be partially regulated by mineralocorticoids.

**Perspectives**

Altered levels of mineralocorticoids may be one factor common to all of the hypertensive rats used in these studies. Excess mineralocorticoid is the basis for DOCA-salt therapy (16), and vessels from SHR rats overexpress the aldosterone synthase gene and overproduce aldosterone (30). Aldosterone plays a role in the development of renal injury in the remnant kidney model of chronic renal failure (13) and is currently receiving renewed attention from hypertension researchers. We are unaware of studies that have measured mineralocorticoid levels in rats treated chronically with NOS inhibitors. However, it could be speculated that chronic inhibition of NO production could lead to enhanced aldosterone synthesis in rats treated with an NOS inhibitor such as l-NAME (14, 18). Abnormal vascular reactivity in SHR stroke prone (SHRSP) has also been thought to be dependent on adrenal mineralocorticoids (2). In support of this concept, plasma aldosterone levels are elevated in 9- and 9-wk-old SHRSP (17), and expression of vascular mineralocorticoid-receptor mRNA is increased in 4- and 9-wk-old SHRSP compared with age-matched WKY (23). Furthermore, spironolactone, a mineralocorticoid-receptor antagonist, has demonstrated a protective effect against cerebrovascular and renal lesions in the absence of any blood pressure-lowering effects in SHRSP compared with placebo-treated SHRSP (20). A recent study found mineralocorticoid hypertensive rats had higher EGF-receptor mRNA levels in cerebral arteries than control normotensive rats, lending support to the idea that mineralocorticoids may regulate EGF levels (9). The ability of mineralocorticoids to alter EGF-receptor expression was confirmed, at least at a molecular and theoretical level, by finding four consensuses mineralocorticoid-response elements in the promoter region of the rat EGF-receptor gene (obtained using GeneBank accession numbers AB025197 and M37394 within MacVector, Genetics Computer Group, Madison, WI). Thus if mineralocorticoids or their receptor levels are enhanced in hypertensive rats, they may, in part, modulate the EGF-receptor signaling pathway via effects on the vasculature.

In summary, these studies determined that arterial contraction to EGF was common to several forms of experimental hypertension. However, contraction to EGF is not solely dependent on a significant elevation in blood pressure, because a dramatic contraction to EGF occurred in vessels from normotensive Wistar-Furth rats on DOCA salt. Thus it appears vascular contraction to EGF may be specifically regulated by mineralocorticoids. Additional experiments examining the influence of mineralocorticoids on the EGF receptor and other proteins associated with the EGF receptor are required to more accurately determine the effect of mineralocorticoids on EGF signaling.

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**REFERENCES**

5. Clegg KB and Sambhi MP. Inhibition of epidermal growth factor-mediated DNA synthesis by a specific tyrosine kinase


