Epidermal growth factor: a potent vasoconstrictor in experimental hypertension

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Florian, Jennifer A., and Stephanie W. Watts. Epidermal growth factor: a potent vasoconstrictor in experimental hypertension. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H976–H983, 1999.—We have tested the hypothesis that growth factor signaling pathways are augmented in hypertension, a disease associated with vascular smooth muscle cell growth. Thoracic aorta was dissected from deoxycorticosterone acetate-salt (DOCA-salt) and one kidney, one clip (1K, 1C) hypertensive rats and from sham normotensive rats for use in isolated tissue bath experiments. Systolic blood pressure was significantly higher in DOCA-salt and 1K, 1C than in normotensive sham rats: 192 ± 7, 185 ± 10, and 117 ± 4 mmHg, respectively. Although virtually no contraction to epidermal growth factor (EGF) was observed in endothelium-denuded sham rat aorta [L = 1% phenylephrine (PE) (10 µmol/l)-induced contraction], the maximal EGF-induced contraction was 47 ± 7% in endothelium-denuded aorta from DOCA-salt hypertensive rats and 39 ± 7% in aorta from 1K, 1C rats. Although slightly attenuated, a contraction to EGF was still observed in endothelium-intact aortic strips from 28-day DOCA-salt hypertensive rats. We also conducted concentration-response curves to EGF on days 1, 3, 5, 7, 14, and 21 of DOCA-salt therapy. A significant contraction to EGF in aorta from DOCA-salt rats was observed on day 14, when DOCA-salt rats had significantly higher blood pressure than sham rats: 188 ± 6 and 122 ± 3 mmHg, respectively. Transforming growth factor-α, an agonist of the EGF receptor, contracted DOCA-salt rat aorta (30 ± 7% PE-induced contraction) but not sham aorta (3 ± 3%). The EGF receptor tyrosine kinase inhibitor 4,5-dianilinophthalimide (10 µmol/l), the mitogen-activated protein kinase kinase inhibitor PD-098059 (10 µmol/l), and the L-type voltage-gated calcium channel inhibitor diltiazem (1 mol/l), but not the cyclooxygenase inhibitor indomethacin (10 µmol/l), virtually abolished EGF-induced contraction (85, 98, and 99% reduction, respectively). These data support a striking difference in EGF signaling between normotensive and hypertensive animals. Furthermore, they provide evidence that growth factors should be considered vasoconstrictors as well as growth modulators in hypertension.

Epidermal growth factor; vascular smooth muscle; tyrosine kinases; contraction; mitogen-activated protein kinase

In humans, epidermal growth factor (EGF) is stored in platelets (19) and, once released, can bind EGF receptors found on vascular smooth muscle (22). The EGF receptor has intrinsic tyrosine kinase activity and is activated via autophosphorylation. Once activated, the receptor is capable of interacting with proteins, including Grb2, Shc, Ras, and Raf, ultimately leading to activation of the tyrosine kinase-dependent extracellular regulated kinase-mitogen-activated protein kinase (Erk-MAPK) pathway. The Erk-MAPK pathway can alter phosphorylation of transcription factors involved in gene expression (20) and stimulate cell proliferation (9, 11) and, more recently, has been implicated in vascular contraction by growth factors and agonists of G protein-coupled receptors (27, 32).

The role of peptide growth factors such as EGF in the vasculature has been considered largely mitogenic. Several studies indicate that aortic smooth muscle cells from spontaneously hypertensive rats (SHR) have increased growth rates or proliferate to a greater extent in response to EGF than normotensive controls (21, 25). In addition, EGF potentiates the mitogenic effects of hormones such as ANG II and vasopressin, hormones established in their interactions with the vasculature (2, 16). One study found that the concentration of genistein, a tyrosine kinase inhibitor, required to reduce EGF-stimulated [H]thymidine incorporation was higher in SHR vascular smooth muscle cells than in cells derived from normotensive Wistar rats (7). These studies suggest that the EGF signaling pathway, which utilizes tyrosine kinases, may be amplified in hypertensive animals.

EGF stimulates contraction in multiple nonvascular tissues from normotensive animals, including guinea pig gastric smooth muscle, raising the possibility that growth factors might influence smooth muscle contraction. A few studies have investigated the effects of EGF in the vasculature (3, 15, 17, 32), but, overall, EGF is an agonist with weak efficacy. No studies have examined the contractile response to EGF in experimental hypertension, and only one has examined the effects of platelet-derived growth factor (PDGF) (23, 24). Because EGF influences vascular reactivity through the activation of tyrosine kinases that are associated with contraction, we hypothesize that the signaling pathway activated by EGF may be upregulated in vessels from hypertensive rats, resulting in a vascular contraction to EGF. In this study we report initial evidence of a novel and dramatic contraction to EGF that occurs in aortic tissue from two forms of experimental hypertension, deoxycorticosterone acetate (DOCA)-salt and the one kidney, one clip (1K, 1C) rat, but not in tissues from normotensive rats. Furthermore, contraction to EGF in these hypertensive vessels is dependent on activation of the EGF receptor, mitogen-activated protein kinase kinase (MEK), and L-type voltage-gated calcium channels, but not the cyclooxygenase pathway.
MATERIALS AND METHODS

Animals. All animal procedures were followed in accordance with institutional guidelines established by Michigan State University. Male Sprague-Dawley rats weighing 300–450 g were purchased from Charles River Laboratories (Portage, MI).

DOCA-salt model of hypertension. Under methoxyflurane (Metophane, Mallinckrodt Veterinary, Mundeldin, IL) anesthesia, the area to be incised was shaved free of fur. The animal’s body temperature was maintained during surgery by a heating pad placed under the rat. 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 subcapular region. Sham rats were uninephrectomized but did not receive the DOCA implant. After surgery, DOCA-treated rats received water supplemented with 1.0% NaCl and 0.2% KCl; sham animals received normal tap water. All animals were fed standard rat chow and had ad libitum access to food and water. After 1, 3, 5, 7, 14, 21, or 28 days, systolic blood pressures (SBP) were measured by slipping a blood pressure cuff on the tail and securing a balloon transducer to the tail. After a stable pulse pressure was obtained, the sphygmomanometer pressure was set to 150–175 mmHg for normotensive rats and 225 mmHg for hypertensive rats to inflate the cuff around the tail. This procedure was repeated three to four times to obtain an average blood pressure for each rat.

Goldblatt 1K,1C model of hypertension. For right uninephrectomy, a mixture of pentobarbital (Nembutal, Abbott Laboratories, N. Chicago, IL; 50 mg/kg ip) and atropine (0.04 mg/kg ip) was administered, and a solid silver clip (0.23 mm ID) was placed around the left renal artery. Sham rats were not uninephrectomized and they did not receive the clip. After surgery the 1K,1C rats were given one analgesic dose of butorphanol tarate (Stadol, Bristol Laboratories, Princeton, NJ; 0.1 mg im). 1K,1C and sham rats were fed standard rat chow and had ad libitum access to food and normal tap water. After 4 wk, SBP was measured using the tail cuff method described above.

Isolated tissue bath protocol. On the appropriate day, rats were killed (80 mg/kg pentobarbital ip) and the thoracic aortas were removed. Arteries were dissected into helical strips (0.25 mm) and the thoracic aortas were removed. Arteries were dissected into helical strips (0.25 mm) and the thoracic aortas were removed. Arteries were dissected into helical strips (0.25 mm), and in most cases the endothelial cell layer was removed by rubbing the luminal side of the vessel with a moistened cotton swab. In experiments examining the contraction to EGF in the presence and absence of the endothelium, the thoracic aorta was cut into two strips; they responded to PE (see Fig. 2 legend). Figure 1 presented isometric contraction to EGF in the presence and absence of the endothelium, the thoracic aorta was cut into two strips; they responded to PE (see Fig. 2 legend).

Tissues were then washed, and the status of the endothelium was examined by observing arterial relaxation to the endothelium-dependent agonist ACh (1 µmol/l) in tissues contracted by a half-maximal concentration of PE (10 µmol/l).

The tissue was incubated with each EGF concentration (10 pmol/l–300 nmol/l) for 10 min before the next concentration was added. The concentration range used in this study is physiologically relevant, inasmuch as normotensive human platelet-rich plasma concentrations of EGF (10–45 pmol/l) have been reported in the low end of this range (19); EGF concentrations in hypertensive humans have not been reported. When the effects of signaling inhibitors on EGF-induced contraction were examined, the vehicle (DMSO, 0.1%) or inhibitor was added to the bath after the tissue had maximally contracted to EGF.

Data analysis. Contractility data (means ± SE) are presented as a percentage of the PE (10–5 mol/l)-induced contraction or the maximal contraction to EGF. Unpaired Student’s t-tests were used where appropriate in comparing two groups’ responses (P < 0.05 considered statistically significant). One-way ANOVA followed by Student-Newman-Keuls comparisons test was used when three or more groups were compared.

Chemicals. Compounds were made in deionized water unless indicated otherwise in parentheses: ACh chloride, atropine, DOCA, diltiazem, indomethacin (DMSO), and PE hydrochloride (Sigma Chemical, St. Louis, MO); 4,5-di-aminophthalimidine (DMSO) (Research Biochemicals International, Natick, MA); EGF (10 nmol/l acetic acid + 1 mg/ml BSA) (GIBCO Life Technologies, Grand Island, NY); transforming growth factor-α (TGF-α) (10 nmol/l acetic acid + 1 mg/ml BSA) (Upstate Biotechnology, Lake Placid, NY). PD-098059 (DMSO) was a kind gift from Dr. David Dudley (Parke Davis, Ann Arbor, MI).

RESULTS

EGF-induced contraction in aorta from DOCA-salt hypertensive and normotensive rats. As represented in Table 1, SBP was significantly higher in 28-day DOCA-salt than in normotensive sham rats: 192 ± 7 and 117 ± 4 mmHg, respectively. Normotensive sham rats weighed significantly more than DOCA-salt rats: 412 ± 8 and 263 ± 9 g, respectively. A dramatic contraction to EGF, 45 ± 7% of a maximal PE (10 µmol/l)-induced contraction, was observed in aortic tissue from rats after 28 days of DOCA-salt therapy, whereas aorta from normotensive sham rats displayed minimal contraction (1 ± 1%) to EGF. The sham aortas were viable, inasmuch as they responded to PE (see Fig. 2 legend). Figure 1 shows a representative tracing of EGF-induced contraction in aorta from a 28-day DOCA-salt hypertensive rat. EGF contracted endothelium-denuded aortic strips from 28-day DOCA-salt rats with a −log EC50 of 7.73 ± 0.73 (µmol/l); an EC50 could not be calculated for the sham rats (Fig. 2, top). This contraction to EGF in aorta from DOCA-salt rats is not merely a shift in the concentration-response curve as seen with 5-hydroxytryptamine (Fig. 2, bottom) but the appearance of a contractile response that is not seen in aorta from normotensive rats. Because contraction to EGF was observed in DOCA-salt aorta but was minimal in aorta from sham normotensive rats, these results suggest that the response may be due to an upregulation in the activity of one or more of the signal transduction pathways.
components activated by the EGF receptor in hypertensive animals.

Having established that this novel contraction to EGF is observable in aorta from 4-wk DOCA-salt hypertensive rats but not in aorta from sham normotensive rats, we wanted to determine when this contractile response first occurred in DOCA-salt rats. Thus sham and DOCA-salt rats were killed on days 1, 3, 5, 7, 14, and 21 of DOCA-salt therapy after measurement of their SBP. There were no differences in the SBP or the contraction to EGF between sham rats on any of the days in this study. On days 1, 3, and 5, there were no differences in the SBP or the contraction to EGF in DOCA-salt and sham rats from day 28 (Table 1, Fig. 3). By day 7, there was a significant difference in the SBP between DOCA-salt and sham rats (153 ± 9 and 116 ± 8 mmHg, respectively) as well as a modest but observable contraction to EGF in aorta from DOCA-salt rats that was not seen in sham rats (Fig. 3). The appearance of a significant contractile response to EGF in aortic strips from DOCA-salt hypertensive rats (57 ± 18% of maximal PE-induced contraction) but not sham normotensive rats (0%) was observed on day 14, when SBP had been elevated for ~1 wk. On day 21 of DOCA-salt therapy, there was a significant difference in SBP between DOCA-salt and sham rats; contraction to EGF in aorta from DOCA-salt rats averaged 29 ± 5% of the maximal PE response compared with 27 ± 25% for sham rats. The standard error for the sham rats is large, because the aorta from one rat had an aberrantly high response to EGF, whereas the others responded minimally. These results suggest that although the vascular effects of EGF on vascular growth and contraction may not contribute to the development of hypertension, the effects may support the chronic maintenance of elevated blood pressure. We are aware that the blood vessels used in this study contribute little to total peripheral resistance, but this finding makes it possible that EGF could exert the same contraction in resistance vessels that regulate blood pressure.

EGF-induced contraction in aorta from 1K,1C hypertensive and normotensive rats. We next determined whether this contractile response to EGF occurred in other forms of experimental hypertension or was specific to the DOCA-salt model of hypertension. Thus we conducted studies investigating EGF-induced contraction in aorta from sham normotensive and 1K,1C hypertensive rats. Although 1K,1C hypertensive rats

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Values are means ± SE of number of animals in parentheses. Epidermal growth factor (EGF) response is expressed as percentage of phenylephrine (PE, 10 µmol/l). SBP, systolic blood pressure; DOCA, deoxycorticosterone acetate; NA, not calculable. Agonist EC50 values, defined as concentration of EGF necessary to produce a half-maximal response, were calculated using a nonlinear regression analysis: effect = maximum response/1 + (EC50/agonist concentration). *Statistical difference (P < 0.05) between SBP of DOCA-salt rats on day 1; †statistical difference (P < 0.05) between SBP of sham rats on day 28; ‡statistical difference (P < 0.05) from maximal response to EGF of sham rats on day 28. The significance of the agonist EC50 values was determined using a one-way ANOVA followed by the Dunnett post-test.
were significantly more hypertensive than the normotensive sham rats (185 ± 10 and 120 ± 2 mmHg, respectively), there was no difference in weight between the two groups (357 ± 12 and 358 ± 15 g, respectively). A significant increase in EGF-induced maximal contraction (39 ± 7%) in aorta from 1K,1C hypertensive rats was observed (Fig. 4). EGF contracted aortic strips with a \(-\log E_{50}\) of 8.80 ± 0.11 mol/l. A small contraction to EGF (7 ± 3%) was observed in aorta from normotensive sham rats with a \(-\log E_{50}\) of 8.62 ± 0.17 mol/l. It is unclear why the 1K,1C sham rats displayed this small but observable contraction to EGF while the DOCA-salt sham rats did not demonstrate a contraction. Both sets of sham rats were purchased from Charles River; however, the 1K,1C rats were not uninephrectomized and were slightly older than the DOCA-salt sham rats. In tissues from 1K,1C and DOCA-salt hypertensive rats, the contraction was slow to develop and was completely reversible. Thus these studies demonstrate a significant difference in EGF signaling between hypertensive and normotensive rats. Indeed, these data indicate that it is not simply a shift in the concentration-response curve to

![Fig. 2. Top: concentration-dependent contraction to EGF in endothelium-denuded aorta from DOCA-salt hypertensive and sham normotensive rats after 28 days of therapy. Contraction to phenylephrine (PE, 10 µmol/l): 844 ± 131 and 799 ± 130 mg for sham and DOCA-salt, respectively. Bottom: concentration-dependent contraction of endothelium-denuded DOCA-salt and sham rat aorta to 5-hydroxytryptamine (5-HT). Contraction to PE (10 µmol/l): 856 ± 138 and 1,060 ± 194 mg in sham and DOCA-salt, respectively. 5-HT curves were conducted in a different set of DOCA-salt rats. Values are means ± SE; n = number of animals. *Statistically significant difference (P < 0.05) between responses of sham and DOCA-salt rat aorta.](http://ajpheart.physiology.org/)

![Fig. 3. Concentration-dependent contraction of endothelium-denuded DOCA-salt hypertensive and sham normotensive rat aorta to EGF on days 1, 3, 5, 7, 14, 21, and 28 of DOCA-salt therapy. Values are means ± SE. Contraction to PE (10 µmol/l): 893 ± 53, 1,050 ± 132, 990 ± 77, 934 ± 55, 951 ± 44, 913 ± 277, and 844 ± 131 mg for days 1, 3, 5, 7, 14, 21, and 28, respectively, in sham rats and 850 ± 13, 980 ± 89, 1,090 ± 30, 1,075 ± 82, 673 ± 93, 770 ± 56, and 799 ± 130 mg, respectively, in DOCA-salt rats.](http://ajpheart.physiology.org/)

EGF but, rather, the appearance of a dramatic concentration-dependent contraction to EGF in hypertensive vessels that was not observed in normotensive vessels.

**Mechanism of EGF-induced contraction in aorta from DOCA-salt hypertensive rats.** With the understanding that endothelium-denuded aortic strips are not entirely physiological, we examined the contraction to EGF in endothelium-intact aortic strips from 28-day DOCA-salt and sham rats to determine whether EGF-induced contraction persisted in the presence of the endothelium. To ensure that the endothelium was intact and functional, ACh (1 µmol/l)-induced vascular relaxation was examined in aortic strips contracted with PE (EC50). Although endothelium-dependent ACh-induced relaxation was known to be reduced in vessels from DOCA-salt hypertensive rats, relaxation was observed when relaxation was observed in endothelium-denuded aortic strips from DOCA-salt hypertensive and sham normotensive rats after 28 days of therapy. Contraction to phenylephrine (PE, 10 µmol/l): 844 ± 131 and 799 ± 130 mg for sham and DOCA-salt, respectively. A small contraction to EGF (7 ± 3%) was observed in aorta from normotensive sham rats with a \(-\log E_{50}\) of 8.62 ± 0.17 mol/l. It is unclear why the 1K,1C sham rats displayed this small but observable contraction to EGF while the DOCA-salt sham rats did not demonstrate a contraction. Both sets of sham rats were purchased from Charles River; however, the 1K,1C rats were not uninephrectomized and were slightly older than the DOCA-salt sham rats. In tissues from 1K,1C and DOCA-salt hypertensive rats, the contraction was slow to develop and was completely reversible. Thus these studies demonstrate a significant difference in EGF signaling between hypertensive and normotensive rats. Indeed, these data indicate that it is not simply a shift in the concentration-response curve to

![Fig. 4. Concentration-dependent contraction of endothelium-denuded sham normotensive and 1 kidney, 1 clip (1K,1C) hypertensive rat aorta to EGF. Contraction to PE (10 µmol/l): 943 ± 32 and 1,172 ± 65 mg for sham and 1K,1C rats, respectively. Values are means ± SE. *Statistically significant difference (P < 0.05) between responses of sham and 1K,1C aorta.](http://ajpheart.physiology.org/)

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in aortic strips from sham (77 ± 6%) and DOCA-salt rats (13 ± 9%), suggesting that the endothelium was intact and somewhat functional. As seen in Fig. 5, contraction to EGF was observed in endothelium-intact (15 ± 10% maximal PE-induced contraction) and endothelium-denuded (28 ± 9%) aortic strips from DOCA-salt rats. A significant difference in the contraction to EGF (1–3 nmol/l) was observed between endothelium-denuded and endothelium-intact aortic strips from DOCA-salt rats. Interestingly, although no contraction was observed in endothelium-denuded sham aorta, a contraction to EGF (12 ± 10% maximal PE-induced contraction) was observed in endothelium-intact aortic strips from sham rats (Fig. 5). These results indicate that a contraction to EGF in aortic strips from DOCA-salt hypertensive rats does occur, even in the presence of the endothelium. In addition, it appears that the endothelium is still somewhat functional given that the response to EGF is slightly blunted in the presence of the endothelium.

Having determined that the contractile response to EGF is dramatically increased in two forms of experimental hypertension, we next examined the signaling pathways utilized by EGF to result in aortic contraction. Inasmuch as the peptide TGF-α is also capable of activating the EGF receptor, we examined the contractile response to TGF-α (10 pmol/l–300 nmol/l) in aortic strips from DOCA-salt and sham rats (Fig. 6). The maximum contractile response to TGF-α was 30 ± 7% of the maximal PE (10 µmol/l)-induced contraction (1,211 ± 141 mg) in vessels from the DOCA-salt rats compared with 3 ± 3% of the PE-induced contraction (1,023 ± 94 mg) in aorta from normotensive sham rats. This experiment suggests that the appearance of contraction is not specific for EGF, but rather the signaling pathway utilized by the EGF receptor may be amplified in DOCA-salt hypertension.

In the next studies, aorta from DOCA-salt rats was maximally contracted to EGF (100–300 nmol/l) and then a signaling inhibitor or vehicle was added for 30 min. EGF binds to a plasma membrane EGF receptor, activating the receptor’s intrinsic tyrosine kinase activity; thus the effect of the EGF receptor tyrosine kinase inhibitor 4,5-dianilinophthalimide (10 µmol/l) on EGF-induced contraction was examined. This concentration of 4,5-dianilinophthalimide, a staurosporine derivative, is documented to reduce significantly EGF-stimulated EGF receptor autophosphorylation (IC₅₀ = 1–10 µmol/l) in serum-starved A-431 cells (4) and, in our laboratory, has minimal effects on phorbol dibutyrate-induced contraction (data not shown). Contraction to EGF was markedly reduced (85% reduction) in the presence of 4,5-dianilinophthalimide (Fig. 7), indicating that EGF-induced contraction requires the activation of the EGF receptor tyrosine kinase. Because EGF utilizes the Erk-MAPK pathway in its mitogenic signal transduction pathway, experiments were conducted to determine whether MEK is activated by EGF to cause contraction. EGF-induced contraction was inhibited 93% by the MEK inhibitor PD-098059 (10 µmol/l; Fig. 7), demonstrating the importance of the dual-specificity (tyrosine and threonine) kinase MEK in EGF-induced vascular contraction.

Vascular smooth muscle contraction is dependent on an increase in intracellular calcium; therefore, EGF-induced smooth muscle contraction was examined in the presence of the L-type voltage-gated calcium channel inhibitor diltiazem (1 µmol/l). A 97% reduction in EGF-induced contraction was observed in the presence of diltiazem (Fig. 7), suggesting that growth factor-induced contraction is also dependent on calcium channels. Some reports in the literature suggest that EGF-induced contraction may be the result of activation of the cyclooxygenase pathway (17); therefore, the effect of indomethacin, a cyclooxygenase inhibitor, on EGF-induced contraction was examined. EGF-induced contraction in the presence of indomethacin (10 µmol/l) was not different from vehicle alone (Fig. 7). This finding indicates that, at least in endothelium-denuded DOCA-salt rat aorta, EGF-induced contraction is not
dependent on activation of the cyclooxygenase pathway. Because EGF-induced contraction is dramatically enhanced in hypertensive vessels and is dependent on tyrosine kinases, these findings suggest that the signaling pathway that utilizes tyrosine kinases may be amplified in hypertension and thus responsible for the contractile response to EGF.

DISCUSSION

This is the first study to demonstrate the appearance of a dramatic contraction to EGF in arteries from two forms of experimental hypertension, the DOCA-salt and 1K,1C models of experimental hypertension, that is not observed in arteries from sham rats. It was also demonstrated that the contraction to EGF is significantly greater in aorta from DOCA-salt rats than in sham aorta on day 14 of therapy, a time when the SBP of DOCA-salt rats is significantly elevated above that of sham rats. Excessive vascular smooth muscle cell growth/remodeling is also common to both models of hypertension used in this study. A significant increase in the total amount of vascular DNA has been observed after 10 days of treatment with deoxycorticosterone-salt therapy compared with sham normotensive rats (14). Moreover, in 1K,1C hypertension the increase in medial smooth muscle mass appears to be due to an increase in smooth muscle volume (cellular hypertrophy) rather than an increase in the number of smooth muscle cells (hyperplasia) (13). Taken together, these data indicate that significant changes in the signaling pathway for EGF occur during the development of hypertension, allowing for the development of a contraction to EGF. Although this change in the vascular reactivity of EGF may not contribute to the development of hypertension, it could be involved in the chronic maintenance of high blood pressure. The authors do recognize that the vessels used in this study contribute little to total peripheral resistance. However, given our results, it is wholly possible that EGF would have similar effects on vascular reactivity in true resistance vessels. Thus EGF should not be considered as only a modulator of vascular growth but also, as we have shown, of vascular reactivity.

With respect to the mechanism by which EGF-induced contraction occurs, we demonstrated that EGF-induced contraction occurs in the presence and absence of the endothelium. Because the response to EGF in the endothelium-intact DOCA-salt strips was slightly attenuated, these findings suggest that the endothelium is still somewhat functional and capable of reducing vascular contraction. Interestingly, a concentration-dependent contraction to EGF was evident in endothelium-intact aortic strips from sham rats but not in endothelium-denuded sham rat aorta. One speculative possibility for the observed contraction to EGF in endothelium-intact aortic strips from sham rats is the presence of endothelial EGF receptors, which, when stimulated, could stimulate the release of an endothelium-derived contractile factor.

Contraction to EGF was observed to be dependent on the activation of calcium channels, inasmuch as the L-type voltage-gated calcium channel inhibitor diltiazem reduced EGF-induced contraction. This finding is not necessarily surprising given the well-established role of calcium in vascular contraction but raises the interesting question, How does a growth factor receptor couple to voltage-gated calcium channels? PDGF increases voltage-operated calcium channel currents in vascular smooth muscle cells isolated from rabbit ear arteries; this increase in calcium channel current appears to require tyrosine phosphorylation (29). Contractile responsiveness to BAY K 8644, a calcium channel agonist, is enhanced in arteries from DOCA-salt rats, suggesting that calcium channel activity is increased in this model of hypertension (28). If the EGF receptor utilizes this same channel or is abnormally coupled to this channel in hypertension, this may be one explanation for the increased efficacy of EGF in vessels from DOCA-salt hypertensive rats. Although previously reported studies indicate that EGF is capable of stimulating calcium mobilization, our findings extend these to demonstrate an important functional role for EGF-induced calcium mobilization, that role being vascular contraction.

In contrast to the crucial role of calcium, activation of the cyclooxygenase pathway does not appear to be a requirement for EGF-induced contraction in aorta from DOCA-salt rats. The cyclooxygenase inhibitor indomethacin did not reduce EGF-induced contraction in DOCA-salt rat aorta. This finding is in disagreement with studies examining EGF-induced contraction in rat ileocolic and superior mesenteric arteries and in guinea pig gastric longitudinal muscle (17, 32). Thus it appears that activation of the cyclooxygenase pathway by EGF may be species and tissue specific.

Because vascular growth and contraction depend on the activation of tyrosine kinases, we contend that the common link in these events may be an upregulation in the activity of tyrosine kinases associated with the EGF.
receptor, and this study provides an initial report on this activity as it relates to contractility. The first tyrosine kinase to be activated by EGF is contained within the EGF receptor. Our results using the EGF receptor tyrosine kinase inhibitor 4,5-dianilinophthalimide indicate that activation of the receptor tyrosine kinase is required for EGF-induced contraction. This is also supported by the finding that contraction to TGF-α, also capable of activating the EGF receptor, was enhanced in strips from DOCA-salt rats. Although it has been suggested that the increased DNA synthesis in SHR cells is due to an increase in the number of EGF receptors and, therefore, increased tyrosine kinase activity associated with those receptors (7), another study found no differences in the number or affinity of EGF receptors for EGF between the SHR and cells from Wistar-Kyoto (WKY) rats (5). In a different study an increase in EGF receptor maximal binding without increased binding affinity in the aorta of the Lyon hypertensive rat was observed compared with control values (26). An increase in the number or binding of EGF to EGF receptors would increase the activation of the receptor tyrosine kinase and the signal transduction pathway associated with the receptor, allowing for an augmented response to EGF, and we are engaged in investigating this possibility.

Studies have reported that growth factor-induced contraction is dependent on the activation of tyrosine kinases (23, 32). MEK is a dual-specificity kinase capable of activating the Erk-MAPK proteins by phosphorylating tyrosine and threonine residues (31). MEK appears to be critical for contraction in response to EGF, inasmuch as the MEK inhibitor PD-098059 dramatically reduced EGF-induced maximal contraction in aorta from DOCA-salt rats. In a different study, aorta from WKY rats and SHR displayed a small, graded contractile response to PDGF, with aorta from SHR achieving a slightly but significantly greater maximal isometric contraction than the WKY rats (225 ± 20 and 153 ± 17 mg, respectively) (23, 24). These studies also reported that basal and PDGF-stimulated tyrosine kinase activity was significantly increased in SHR compared with WKY aorta. Interestingly, in acute hypertension the Erk-MAPK proteins have been shown to be upregulated in response to a rise in blood pressure (30). An upregulation of MAPK proteins could result in increased phosphorylation of contractile proteins. Several studies have shown that MAPK is capable of associating with (12) and phosphorylating caldesmon (1, 6, 10), thereby reversing its inhibitory activity on actinomyosin ATPase and allowing smooth muscle contraction to occur (18). Another contractile protein, myosin light chain, has been reported to be phosphorylated on treatment with fetal calf serum, which is known to contain several tyrosine kinase-dependent growth factors. Moreover, the resultant phosphorylation and contraction could be inhibited by the tyrosine kinase inhibitor genistein (8). Collectively, these studies further support the idea that a contractile response to EGF may be the result of increased tyrosine kinase activity associated with the EGF receptor.

Finally, it should be noted that the concentrations of growth factors used in these studies are in a range for exerting physiological modifications on blood vessels that could affect total peripheral resistance (19). This idea is especially compelling given the knowledge that, in humans, EGF is stored in platelets along with other contractile agonists and mitogens, such as 5-hydroxytryptamine. Thus it can be envisioned that during a thrombotic event leading to platelet degranulation and the release of platelet contents the potential exists for synergistic action of these vascular agonists on not only growth but contraction.

In summary, these data suggest that the signal transduction elements for EGF, possibly including the tyrosine kinase(s) associated with the EGF receptor, may be enhanced in hypertension, resulting in an augmented contractile response to EGF. Contraction to EGF is dependent on activation of the EGF receptor tyrosine kinase MEK and L-type calcium channels and persists in the presence of the endothelium. Additional studies measuring actual tyrosine kinase activity in normotensive and hypertensive vessels may possibly reveal a potentially therapeutic use for specific tyrosine kinase inhibitors to reduce blood pressure via inhibition of not only vascular smooth muscle growth but also contraction.

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