Epidermal growth factor (EGF) is not only a cell mitogen but a potent vasoconstrictor that shares many properties with angiotensin II. Because EGF is localized in the kidney, we have studied the direct effects of EGF on renin secretion using both static incubations and perifusions of rat renal cortical slices. EGF at \(5 \times 10^{-9} \text{M}\) significantly inhibited renin secretion in static incubations (control, 100±2%; EGF, 72±3%; \(p<0.001\)). When added to perifusions, EGF acted rapidly, reducing renin secretion at the earliest time period (10 minutes). Similarly, transforming growth factor-\(\alpha\), which can bind to the EGF receptor, also inhibited renin secretion (control, 92±8%; transforming growth factor-\(\alpha\) \([2 \times 10^{-7} \text{M}]\), 63±4%; \(p<0.02\)). Because both prostaglandins and lipoxigenase products of arachidonic acid have been shown to play a role in some EGF-mediated actions, we examined these possible mechanisms of EGF action. Meclofenamate, a cyclooxygenase blocker, and BW755c and baicalein, both lipoxigenase blockers, were studied. None of these agents altered EGF-mediated renin inhibition. EGF action has also been coupled to the stimulation of tyrosine kinase activity; therefore, we examined the effects of the tyrosine kinase inhibitors genistein and quercetin. Both genistein \((10^{-5} \text{M})\) and quercetin \((10^{-5} \text{M})\) abolished the inhibition of renin by EGF (control, 100±3%; EGF, 82±4%; EGF plus genistein, 110±7%; \(p<0.01\); EGF, 75±4%; EGF plus quercetin, 92±4%; \(p<0.01\)). These results suggest that EGF is a potent inhibitor of renin release and that eicosanoids do not play a role in EGF action. However, blockade of tyrosine kinase activity prevents EGF inhibition of renin secretion. These studies support a potential paracrine role of EGF in renin secretion.

Methods

Renal cortical slices from rats (male Sprague-Dawley, weighing 150–250 g, 42–54 days old) were used for both static incubations and perifusion experiments (Endotronics Acusyst S perifusion system, Endotronics, Inc., Marietta, Ohio) with a method described previously. Briefly, two slices (approximately 0.5 mm thick) were obtained from the superficial cortex of each kidney with a Stadie-Riggs microtome. For static incubations, slices (15–30 mg) were washed twice with Krebs-Ringer bicarbonate with glucose (KRBG) medium containing 0.2% bovine serum albumin. The washed slices were preincubated in a metabolic shaker, saturated with 95% \(O_2–5% \text{CO}_2\), at 37°C for 30 minutes, and then incubated for two consecutive 30-minute incubation periods. The medium was changed during wash periods and after the preincubation and incubation periods. Each slice was incubated for a 30-minute baseline period, after which various agents were added. The response to an agent was observed for the next 30-minute period, thus enabling each slice to serve as its own control. The standard KRBG medium contained (mM): \(\text{NaCl} 120, \text{KCl} 4.7, \text{MgSO}_4 1.2, \text{CaCl}_2 2.5, \text{KH}_2\text{PO}_4 1.2, \text{NaHCO}_3 26.8, \text{and glucose} 10, \text{pH} 7.4\). For perifusions, slices were placed in culture chambers and perifused with KRBG buffer at a flow rate of 0.5 mL/min, as described.
Effects of Epidermal Growth Factor on Renin Release

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Effects of Epidermal Growth Factor on Renin Release

In static incubations, renin release from individual cortical slices was relatively stable during two consecutive 60-minute incubation periods. In a representative experiment, the mean renin release during the first 30 minutes of incubation was 22.5 ng Ang I/mg tissue per hour (100%) and during the second 30 minutes of incubation was 21.6 ng Ang I/mg tissue per hour (96%). However, the absolute levels of renin release exhibit considerable variation between incubations even when corrected for slice weight, as indicated previously by us and others, but values within the same slice do not greatly differ. This finding emphasizes the importance of using each slice as its own control.

In static incubations, EGF at a concentration of $5 \times 10^{-10}$ M did not alter renin significantly (97±3%) compared with controls (99±2%), but $2 \times 10^{-9}$ M EGF decreased renin levels to 80±8%, and $5 \times 10^{-8}$ M EGF reduced renin release to 72±3% of basal values ($p<0.001$) (Figure 1 and Table 1). A higher concentration ($10^{-7}$ M) did not further inhibit renin release (73±6%).

We compared the renin-inhibitory action of EGF with Ang II in a static incubation system. Ang II produced a dose-related decrease in renin secretion (basal, 100±2%; $10^{-10}$ M, 96±7%; $10^{-9}$ M, 91±3%; $10^{-8}$ M, 79±4%; $p<0.001$). A higher concentration of Ang II ($10^{-7}$ M) did not further decrease renin release (78±4%).

To fully evaluate the more definitive role of EGF and its time course, we performed perfusion studies. Control slices released renin in a relatively stable manner over the entire time period (60 minutes) (Figure 2). EGF ($5 \times 10^{-8}$ M) caused a significant inhibition of renin secretion, and its action was evident as early as 10 minutes. The maximal inhibition of EGF was seen at 30 minutes after EGF perfusion was started.

Effects of Transforming Growth Factor-α on Renin Release

TGF-α also inhibited renin secretion. As little as $2 \times 10^{-9}$ M significantly decreased renin release to 63±4% of the control value ($p<0.02$). A higher concentration of $4 \times 10^{-9}$ M further decreased renin levels to 55±5% ($p<0.001$) (Figure 3).

Effects of Meclofenamate, BW755c, and Baicalein on Epidermal Growth Factor–Induced Renin Inhibition

We used meclofenamate as the cyclooxygenase blocker, because indomethacin may have some nonspecific inhibitory actions. Meclofenamate (50 µM) by itself had no significant effect on basal renin secretion. Similarly, both lipoxygenase blockers, BW755c ($10^{-3}$ M) and baicalein ($10^{-6}$ M) did not alter basal renin secre-
TABLE 1. Renin Release in Control Slices and After Addition of Epidermal Growth Factor

<table>
<thead>
<tr>
<th>Slice</th>
<th>Control (ng Ang I/mg tissue per hour)</th>
<th>After EGF (ng Ang I/mg tissue per hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 minutes</td>
<td>30 minutes</td>
</tr>
<tr>
<td>1</td>
<td>18.3</td>
<td>18.8</td>
</tr>
<tr>
<td>2</td>
<td>11.0</td>
<td>10.2</td>
</tr>
<tr>
<td>3</td>
<td>5.8</td>
<td>5.9</td>
</tr>
<tr>
<td>4</td>
<td>29.1</td>
<td>29.4</td>
</tr>
<tr>
<td>5</td>
<td>14.4</td>
<td>13.3</td>
</tr>
<tr>
<td>6</td>
<td>34.5</td>
<td>35.2</td>
</tr>
<tr>
<td>7</td>
<td>10.2</td>
<td>8.6</td>
</tr>
<tr>
<td>8</td>
<td>21.0</td>
<td>22.1</td>
</tr>
<tr>
<td>9</td>
<td>11.4</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Mean±SEM  17.3±3.2  17.2±3.3  18.0±3.4  12.7±2.2

Ang I, angiotensin I; EGF, epidermal growth factor.
*p<0.001 compared with respective control value.

tion significantly.20 As shown in Figure 4, neither meclofenamate, BW755c, nor baicalein altered EGF-induced renin inhibition.

Effects of Genistein and Quercetin on Epidermal Growth Factor Action

We studied the effects of two putative tyrosine kinase inhibitors, genistein and quercetin.21 Genistein at a concentration of 10^-5 M did not alter basal renin release (100±3%). However, this dose of genistein (10^-5 M) significantly blocked EGF inhibition of renin secretion (110±7% versus 82±4%, p<0.01) (Figure 5). Similarly, quercetin, a structurally distinct tyrosine kinase inhibitor, also blocked the EGF inhibition of renin release (92±4% versus 75±4%, p<0.01) (Figure 6). Quercetin at a concentration of 10^-6 M by itself did not alter basal renin secretion.

Effect of Genistein on Phorbol 12-Myristate 13-Acetate–Induced Renin Release

To determine the specificity of genistein, we examined the role of genistein on TPA-induced renin release.

TPA at a concentration of 10^-5 M has previously been shown to inhibit renin secretion.23 When slices were incubated with 10^-5 M TPA, they released less renin (79±4%) than control slices (105±9%, p<0.01).23 However, unlike the EGF experiments, genistein did not alter TPA-induced renin inhibition (Figure 7).

Discussion

The present studies represent the first demonstration of an effect of EGF on renin secretion. These effects of EGF on renin inhibition are seen at a concentration as low as 5x10^-9 M. These EGF concentrations are 100-fold higher than those reported to be present in human whole blood.24 However, only small amounts of EGF exist free in circulation, as most is associated with platelets,24 and one might anticipate that higher local concentrations would be available at the sites of paracrine/autocrine production in the kidney.10,11 Ang II is a

FIGURE 2. Line graph shows time course and effects of epidermal growth factor (EGF) on renin secretion in perfused rat renal cortical slices. Perifusions were performed as indicated in text. Results are expressed as percentage of each basal renin release during first 0–10 minutes. Values represent mean±SEM of six to seven different experiments. EGF significantly inhibits renin secretion and its action, evident as early as 10 minutes.

FIGURE 3. Bar graph shows effects of transforming growth factor-α (TGF-α) on renin release in rat renal cortical slices at 30 minutes. Values (percentage of control) are mean±SEM, representing five to eight experiments. *p<0.02, **p<0.001 vs. control.
major physiological negative feedback regulator of renin secretion. We compared the effects of EGF with those of Ang II in similar studies and found that EGF was more potent than Ang II in its action. The EGF concentrations used here, however, are similar to the concentrations that induce other well-established in vitro effects such as stimulation of cortisol production in ovine adrenal cells or steroidogenesis in Leydig's cells.25-26 The effects of EGF were similar in both the static incubation system and the perfusion system. In the latter case, EGF action on renin was rapid (10 minutes). Similarly, EGF-induced contractions and calcium efflux in rat aorta4 are also seen within minutes (3–5 minutes). Other rapid effects of EGF include tyrosine kinase activation, increased prostaglandin production, and stimulation of Na⁺-H⁺ exchange.27 TGF-α shares significant sequence homology with EGF; both are processed from larger transmembrane protein precursors and both are believed to bind to and activate the EGF receptor.28 TGF-α was found to be slightly more effective than EGF in modulating renin inhibition. Similarly, TGF-α has been shown to be more potent than EGF in induction of angiogenesis,29 promotion of wound healing,30 and stimulation of calcium release from bone.31 However, the basis for the different potencies of the two ligands is not known, and few comparative data on receptor binding properties are available. It has been suggested that TGF-α and EGF may interact with their common receptor in nonidentical ways.28 Studies by Decker31 indicate that EGF directs the EGF receptor directly to a degradative pathway, whereas TGF-α allows receptor recycling before degradation. This may explain some of the results in which TGF-α functions as a superagonist relative to EGF.

We then investigated the possible mechanism or mechanisms of EGF action. The intracellular signals involved in EGF action in a number of systems have been reported to be similar to those for Ang II. EGF, like Ang II, stimulates phospholipase C; induces phosphatidylinositol turnover; increases diacylglycerol, an activator of protein kinase C; and induces increases in cytosolic calcium levels.4-6 Some recent evidence indicates that phosphatidylethanolamine may also be a source of EGF-induced diacylglycerol production.32 Because diacylglycerol is rich in arachidonic acid, it is a major contributor to cyclooxygenase and lipoxygenase products. EGF has been shown to stimulate prostaglandin synthesis in rat mesangial cells,7 in canine kidney cells,8 and in amnion cells.9 Lipoxygenase products have also been shown to play a role in EGF-induced action. For example, EGF-mediated uterine contractions can be blocked by lipoxygenase inhibitors,33 and EGF-induced human chorionic gonadotropin secretion is potentiated by 15-lipoxygenase product.34 We have previously shown that lipoxygenase-derived prod-
mimicked by certain peptide growth factors. These studies point to a potential paracrine role of EGF in renin secretion, and this could be a factor in some low renin states such as in hyporeninemic hypoaldosteronism.

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