Renal microvascular actions of calcitonin gene-related peptide

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Renal microvascular actions of calcitonin gene-related peptide. Am. J. Physiol. 274 (Renal Physiol. 43): F1078–F1085, 1998.—Calcitonin gene-related peptide (CGRP) is a potent vasodilator that is suggested to act via ATP-sensitive K channels (K\(_{\text{ATP}}\)). In the present study, we examined the actions of CGRP on pressure- and angiotensin II-induced vasoconstriction, using the in vitro perfused hydronephrotic rat kidney. Elevated pressure (from 80 to 180 mmHg) and 0.1 nM angiotensin II elicited similar decreases in afferent diameter in this model. CGRP inhibited myogenic reactivity in a concentration-dependent manner, completely preventing pressure-induced constriction at 10 nM (95 ± 10% inhibition). These effects were partially attenuated by 10 µM glibenclamide (62 ± 16% inhibition, P = 0.025), indicating both K\(_{\text{ATP}}\)-dependent and -independent actions of CGRP. In contrast, 10 nM CGRP inhibited angiotensin II-induced vasoconstriction by only 54 ± 11%, and this action was not affected by glibenclamide (41 ± 11%, P = 0.31). CGRP also inhibited the efferent arteriolar response to angiotensin II in the absence and presence of glibenclamide. Pinacidil (1.0 µM), a K\(_{\text{ATP}}\) opener also preferentially inhibited pressure- vs. angiotensin II-induced vasoconstriction (97 ± 5 and 59 ± 13% inhibition, respectively; P = 0.034). We conclude that the renal vasodilatory mechanisms of CGRP are pleiotropic and involve both K\(_{\text{ATP}}\)-dependent and -independent pathways. The effectiveness of CGRP in opposing renal vasoconstriction and the role of K\(_{\text{ATP}}\) in this action appear to depend on the nature of the underlying vasoconstriction. We suggest that this phenomenon reflects an inhibition of K\(_{\text{ATP}}\) activation by angiotensin II.

CALCITONIN GENE-RELATED PEPTIDE (CGRP) is a potent vasodilator and neurotransmitter that may contribute to the regulation of renal hemodynamics. Nerve endings containing CGRP have been identified on the renal vasculature (21), especially in the vicinity of glomerulus (22, 36). In vivo studies confirm a potential role of CGRP in the regulation renal blood flow and renal function, as CGRP administration is associated with both direct and indirect effects on renal vascular resistance and glomerular filtration rate (reviewed in Ref. 39). Few studies have directly examined the actions of CGRP on renal microvessels. Edwards and Trizina (8), using pressurized renal arterioles isolated from the rabbit, reported that CGRP reverses norepinephrine-induced afferent arteriolar vasoconstriction but does not affect the efferent arteriolar vasoconstrictions induced by norepinephrine or angiotensin II. Although these observations suggest that CGRP is an important renal vasodilator, the characteristics of CGRP-induced renal vasodilation are not well defined, and the cellular mechanisms mediating this action have not been determined.

CGRP activates adenylate cyclase and elevates cellular levels of cAMP in a number of cell types, including renal tissue (9). The signaling cascade and the final transduction mechanisms mediating the vasodilatory actions of CGRP, however, are controversial. In some experimental settings, CGRP activates ATP-sensitive potassium channels (K\(_{\text{ATP}}\)) via stimulation of cAMP-dependent protein kinase (reviewed in Refs. 32, 35). K\(_{\text{ATP}}\) is an important modulator of the renal microvasculature (28), and we have previously shown that activation of K\(_{\text{ATP}}\) preferentially attenuates the reactivity of the afferent arteriole (37). However, in many preparations, the actions of CGRP are insensitive to glibenclamide and clearly involve mechanisms other than K channel-induced hyperpolarization (5). The involvement of K\(_{\text{ATP}}\) in the renal microvascular actions of CGRP has not been previously investigated, and both species and regional variations in the mechanisms mediating CGRP-induced vasodilation are documented (3). Finally, the nature of the underlying tone (e.g., myogenic- vs. agonist-induced vasoconstriction) may influence CGRP-induced vasodilation. Vasoconstrictor agonists have been shown to directly inhibit K\(_{\text{ATP}}\) in isolated myocytes (4) and could potentially modify the actions of vasodilators acting through this channel.

The present study was initiated to test the hypothesis that CGRP elicits vasodilation by activating K\(_{\text{ATP}}\) in the renal microvasculature. We also compared the actions of CGRP during pressure- and angiotensin II-induced vasoconstriction. Our findings indicate that CGRP acts, in part, by a K\(_{\text{ATP}}\)-dependent mechanism in the kidney but also suggest that the nature of the underlying renal vascular tone influences the vasodilatory mechanisms of CGRP. Specifically, we found that glibenclamide attenuates the effects of CGRP during myogenic vasoconstriction but not during angiotensin II-induced vasoconstriction, a finding consistent with previous in vitro observations indicating that angiotensin II inhibits the activation of K\(_{\text{ATP}}\) in isolated vascular myocytes (20).

MATERIAL AND METHODS

The isolated hydronephrotic rat kidney model was used to examine the renal microvascular actions of CGRP. Young male Sprague-Dawley rats (100–150 g) were anesthetized (halothane), and the left ureter was surgically ligated to induce unilateral hydronephrosis. After 5–8 wk, the hydronephrotic kidneys were harvested. The left renal artery supplying the hydronephrotic kidney was canulated, the kidney was excised, the renal capsule was removed, and the kidney
was transferred to the stage of an inverted microscope. During the initial cannulation and throughout the excision and transfer, kidneys were continuously perfused at 80 mmHg to preserve nutritive flow and to avoid hypoxia or ischemia.

The perfusing apparatus used in the present study employed a single-pass presentation of medium to the kidney (37). The medium was pumped on demand through a heat exchanger to a pressurized reservoir, supplying the renal artery. Perfusion pressure was monitored within the renal artery and controlled by adjusting the pressure within the reservoir. Kidneys were perfused at 80 mmHg with modified Dulbecco's medium containing (in mM) 30 bicarbonate, 5 glucose, 1 sodium pyruvate, and 5 HEPES. The perfusate was equilibrated with 95% air-5% CO₂ (P O₂ = 150 mmHg). Temperature and pH were maintained at 37°C and 7.40, respectively. Drugs are added directly to the perfusate but reach the tissues from both the luminal and abluminal sides, as the renal venous effluent bathes the kidney.

Kidneys were perfused on a heated stage of an inverted microscope. A small region of the transparent renal cortex was transilluminated and stabilized with a fiber light guide. Diameters were measured from the digitized video image (model IVG-128; Datacube, Peabody, MA), using custom software described previously (25). Afferent arteriolar diameters were measured in the most proximal regions, just after branching from the interlobular artery. Efferent arteriolar diameters were measured within 50 µm of the glomerulus.

Kidneys were allowed to equilibrate for at least 30 min. In the first series, stable myogenic responses were obtained to two to three consecutive pressure ramps (from 60 to 180 mmHg in 20-mmHg increments). Kidneys were then treated with increasing concentrations of CGRP added directly to the perfusate. After allowing at least 10-min equilibration at each concentration, the response to pressure was reassessed. In a separate group of kidneys, the effects of CGRP were assessed following pretreatment with 10 µM glibenclamide. Glibenclamide is quite selective for K_ATP at concentrations of 1–10 µM and is widely used to assess the involvement of this channel in smooth muscle mechanisms (28, 35). We chose the higher concentration to facilitate interpretations of negative results. Glibenclamide blocks Cl currents and Ca-activated K channels at even higher concentrations (35), but our observation that 10 µM did not affect basal diameters or vasoconstrictor responses (see RESULTS) militates against such possible actions in the present study.

In the second series, 0.1 nM angiotensin II was administered at a constant pressure (80 mmHg), and afferent and efferent arteriolar diameters were measured from the same glomerulus. After washout of the angiotensin II, kidneys were treated with increasing concentrations of CGRP and the responses to angiotensin II were reassessed. In a separate group, kidneys were pretreated with 10 µM glibenclamide, and the identical protocol was followed. In the last series of experiments, myogenic responses were assessed in controls and following increasing concentrations of pinacidil. These data were compared with previous findings assessing the actions of pinacidil on angiotensin II-induced vasoconstriction (37). In all experiments, ibuprofen (10 µM) was included in the perfusate to eliminate the effects of renal prostaglandins.

Ibuprofen and glibenclamide were obtained from Research Biochemicals International (Natick, MA). Angiotensin II and calcitonin gene-related peptide were obtained from Sigma (St. Louis, MO). All other reagents were obtained from Life Technologies (Grand Island, NY).

Data are expressed as means ± SE as an index of dispersion. The number of replicates refers to the number of afferent and/or efferent arterioles examined. One afferent arteriole or one pair of afferent and efferent arterioles were studied in each kidney preparation. Differences between means were evaluated by analysis of variance followed by Student’s t-test (paired or unpaired). Multiple comparisons were analyzed by the Newman-Keuls test. P < 0.05 was considered statistically significant.

RESULTS

Figure 1A illustrates the effects of CGRP on pressure-induced afferent arteriolar vasoconstriction. In the
absence of CGRP, afferent arteriolar diameters were 16.7 ± 0.9, 16.5 ± 1.0, and 13.8 ± 0.7 µm at 60, 80, and 100 mmHg, respectively. Significant vasoconstrictions were elicited at perfusion pressures of 120 mmHg and higher. Diameters decreased to 11.8 ± 0.9, 9.6 ± 1.0, 8.9 ± 1.3, and 8.2 ± 1.2 µm at 120, 140, 160, and 180 mmHg, respectively (P < 0.05 vs. 60 mmHg for all values). Pretreatment with CGRP (0.001–10 nM) did not alter afferent arteriolar diameter at 60 or 80 mmHg (P > 0.05 vs. control, n = 6) but, as depicted in Fig. 1A, inhibited the afferent arteriolar response to elevated pressure in a concentration-dependent manner, completely abolishing the response at 10 nM. At 10 nM CGRP, afferent arteriolar diameters were 16.1 ± 0.9, 15.0 ± 0.7 µm (60 mmHg) and 15.0 ± 0.7 µm (80 mmHg) to 11.7 ± 1.0, 9.9 ± 1.1, 8.6 ± 0.9, 7.5 ± 0.9, and 7.0 ± 0.8 µm at 100, 120, 140, 160, and 180 mmHg, respectively (P < 0.05, n = 9), in preparations pretreated with 10 µM glibenclamide. Thus the threshold pressure for myogenic vasoconstriction appeared slightly reduced in this group. In a previous study using a paired experimental design (28), glibenclamide had no significant effect on basal myogenic reactivity. CGRP (0.001–10 nM) had no effect on basal diameter at either 60 or 80 mmHg (P > 0.05 vs. control, n = 9) but shifted the threshold for pressure-induced vasoconstriction to 140 and 160 mmHg, respectively, at 0.1 and 1.0 nM. Only at 10 nM did CGRP significantly inhibit myogenic vasoconstriction at all pressures. In the presence of glibenclamide and 10 nM CGRP, afferent arteriolar diameters were 16.1 ± 0.6, 16.5 ± 0.6, 15.5 ± 1.1, 14.8 ± 1.5, 13.8 ± 1.8, 13.1 ± 1.7, and 12.6 ± 1.8 µm at 60, 80, 100, 120, 140, 160, and 180 mmHg, respectively.

Glibenclamide partially attenuated these actions of CGRP (Fig. 1B). In the absence of CGRP (control), mean afferent arteriolar diameter decreased from 15.2 ± 0.7 µm (60 mmHg) and 15.0 ± 0.7 µm (80 mmHg) to 11.7 ± 1.0, 9.9 ± 1.1, 8.6 ± 0.9, 7.5 ± 0.9, and 7.0 ± 0.8 µm at 100, 120, 140, 160, and 180 mmHg, respectively (P < 0.05, n = 9), in preparations pretreated with 10 µM glibenclamide. Thus the threshold pressure for myogenic vasoconstriction appeared slightly reduced in this group. In a previous study using a paired experimental design (28), glibenclamide had no significant effect on basal myogenic reactivity. CGRP (0.001–10 nM) had no effect on basal diameter at either 60 or 80 mmHg (P > 0.05 vs. control, n = 9) but shifted the threshold for pressure-induced vasoconstriction to 140 and 160 mmHg, respectively, at 0.1 and 1.0 nM. Only at 10 nM did CGRP significantly inhibit myogenic vasoconstriction at all pressures. In the presence of glibenclamide and 10 nM CGRP, afferent arteriolar diameters were 16.1 ± 0.6, 16.5 ± 0.6, 15.5 ± 1.1, 14.8 ± 1.5, 13.8 ± 1.8, 13.1 ± 1.7, and 12.6 ± 1.8 µm at 60, 80, 100, 120, 140, 160, and 180 mmHg, respectively.

Fig. 1. Time and application control data illustrating that reproducible afferent and efferent arteriolar responses to angiotensin II are obtained with this model. Solid, dotted, and dashed lines represent first, second, and third applications of 0.1 nM angiotensin II (n = 4).

Fig. 2. Summary illustrating the effects of glibenclamide on CGRP-induced inhibition of afferent arteriolar myogenic vasoconstriction. Percent inhibition of myogenic vasoconstriction were calculated for pressures of 140–180 mmHg and combined. CGRP exerted a significantly reduced effect in the glibenclamide-treated group (C). Values are means ± SE. *P < 0.05 vs. control (data taken from Fig. 1).
Thus 10 nM CGRP only partially inhibited the vasoconstrictor response to angiotensin II, whereas this concentration completely prevented pressure-induced afferent arteriolar vasoconstriction (Fig. 2). As previously reported (37), 10 µM glibenclamide did not alter basal afferent and efferent arteriolar diameters or affect the responses to angiotensin II. In a paired series (n = 5), basal afferent arteriolar diameters were 15.4 ± 1.4 µm in the absence and 15.1 ± 1.2 µm in the presence of glibenclamide (P > 0.5), and angiotensin II (0.1 nM) decreased diameters to 7.6 ± 1.5 and 7.0 ± 1.3 µm in the absence and presence of glibenclamide, respectively (P > 0.2). Basal efferent arteriolar diameters were 12.6 ± 0.9 µm in the absence and 11.7 ± 1.1 µm in the presence of glibenclamide (P > 0.05) and decreased to 6.6 ± 1.0 and 5.7 ± 0.8 µm in the absence and presence of glibenclamide, respectively (P > 0.2), upon treatment with angiotensin II.

The effects of glibenclamide on the actions of CGRP during angiotensin II-induced vasoconstriction are summarized in Table 2. In the afferent arteriole, 10 nM CGRP significantly attenuated the angiotensin II response (P < 0.05 vs. 0 CGRP), whereas, in the efferent arteriole, the attenuation failed to reach statistical significance (P > 0.05 vs. 0 CGRP). The data from Tables 1 and 2 were expressed as percent inhibition of angiotensin II-induced vasoconstriction to more critically assess the effects of glibenclamide. In the afferent arteriole, 10 nM CGRP inhibited the angiotensin II responses by 53 ± 11% in the absence and by 41 ± 11% in the presence of glibenclamide (P = 0.42, n = 8). The corresponding values for the efferent arteriole were 33 ± 13% and 39 ± 14% (P = 0.77, n = 8). Thus CGRP attenuated the angiotensin II-induced vasoconstriction in a similar manner in either the absence or presence of 10 µM glibenclamide in both vessels, suggesting that activation of K_ATP does not contribute to the actions of CGRP in this setting.

The inhibitory actions of 10 nM CGRP on pressure- and angiotensin II-induced afferent arteriolar responses are plotted as percent inhibition of vasoconstriction in Fig. 4A. As shown in Fig. 4B, 0.1 nM angiotensin II and elevated pressure (from 80 to 180 mmHg)

### Table 1. Effects of CGRP on vasoconstrictor responses of afferent and efferent arterioles to angiotensin II

<table>
<thead>
<tr>
<th>CGRP</th>
<th>Control</th>
<th>ANG II (10⁻¹⁰ M)</th>
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<tbody>
<tr>
<td></td>
<td>Afferent arteriolar diameter, µm</td>
<td></td>
</tr>
<tr>
<td>0 M</td>
<td>16.3 ± 0.5</td>
<td>6.1 ± 0.6*</td>
</tr>
<tr>
<td>10⁻¹⁰ M</td>
<td>16.0 ± 0.5</td>
<td>7.0 ± 0.5*</td>
</tr>
<tr>
<td>10⁻⁹ M</td>
<td>16.4 ± 0.6</td>
<td>9.6 ± 1.3*</td>
</tr>
<tr>
<td>10⁻⁸ M</td>
<td>16.3 ± 0.8</td>
<td>11.5 ± 1.2†</td>
</tr>
<tr>
<td></td>
<td>Efferent arteriolar diameter, µm</td>
<td></td>
</tr>
<tr>
<td>0 M</td>
<td>11.8 ± 1.1</td>
<td>4.6 ± 0.5*</td>
</tr>
<tr>
<td>10⁻¹⁰ M</td>
<td>11.5 ± 1.0</td>
<td>4.5 ± 1.7*</td>
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<tr>
<td>10⁻⁹ M</td>
<td>12.0 ± 1.1</td>
<td>5.2 ± 1.4*</td>
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<tr>
<td>10⁻⁸ M</td>
<td>11.7 ± 1.1</td>
<td>7.1 ± 1.0†</td>
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Values are means ± SE, n = 8. CGRP, calcitonin gene-related peptide. *P < 0.05 vs. control. †P < 0.05 vs. 0 CGRP.

### Table 2. Effects of CGRP on vasoconstrictor responses of afferent and efferent arterioles to angiotensin II in the presence of 10 µM glibenclamide

<table>
<thead>
<tr>
<th>CGRP</th>
<th>Control</th>
<th>ANG II (10⁻¹⁰ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Afferent arteriolar diameter, µm</td>
<td></td>
</tr>
<tr>
<td>0 M</td>
<td>12.5 ± 0.8</td>
<td>4.7 ± 0.8*</td>
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<tr>
<td>10⁻¹⁰ M</td>
<td>13.0 ± 0.6</td>
<td>5.0 ± 1.0*</td>
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<tr>
<td>10⁻⁹ M</td>
<td>12.9 ± 0.7</td>
<td>5.2 ± 1.0*</td>
</tr>
<tr>
<td>10⁻⁸ M</td>
<td>14.7 ± 0.6</td>
<td>9.0 ± 1.1†</td>
</tr>
<tr>
<td></td>
<td>Efferent arteriolar diameter, µm</td>
<td></td>
</tr>
<tr>
<td>0 M</td>
<td>9.2 ± 0.8</td>
<td>5.5 ± 0.5*</td>
</tr>
<tr>
<td>10⁻¹⁰ M</td>
<td>9.2 ± 0.7</td>
<td>5.1 ± 0.6*</td>
</tr>
<tr>
<td>10⁻⁹ M</td>
<td>9.1 ± 0.6</td>
<td>5.2 ± 0.6*</td>
</tr>
<tr>
<td>10⁻⁸ M</td>
<td>9.0 ± 0.7</td>
<td>6.7 ± 0.7*</td>
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Values are means ± SE, n = 8. *P < 0.05 vs. control. †P < 0.05 vs. 0 CGRP.

Fig. 4. Comparison of vasodilatory actions of 10 nM CGRP during pressure-induced (myogenic, closed bars) vs. angiotensin II-induced (open bars) vasoconstriction in the absence (control, A, left) and presence of 10 µM glibenclamide (A, right). CGRP caused a significantly greater inhibition of myogenic vasoconstriction in controls. This difference was abolished by glibenclamide. B: studies were conducted at comparable levels of myogenic and angiotensin II-induced vasoconstriction. Values are means ± SE.
elicited similar degrees of afferent arteriolar vasoconstriction in both the absence and presence of glibenclamide. In the absence of glibenclamide (Fig. 4A, control), 10 nM CGRP caused a greater inhibition of myogenic vs. angiotensin II-induced vasoconstriction (95 ± 10 vs. 53 ± 11%, respectively; P = 0.025). Pretreatment with glibenclamide (Fig. 4A) eliminated this difference (62 ± 16 and 41 ± 11% inhibition of myogenic and angiotensin II responses, respectively; P = 0.31). These data suggest that the differing effects of CGRP on pressure- vs. angiotensin II-induced vasoconstriction are due to a glibenclamide-sensitive (K<sub>ATP</sub> mediated) component of CGRP-induced vasodilation that is observed during pressure-induced vasoconstriction but is not seen when vasoconstriction is induced by angiotensin II.

The above findings suggest that either the activation of K<sub>ATP</sub> or the effectiveness of K<sub>ATP</sub>-induced hyperpolarization differ during angiotensin II- vs. pressure-induced afferent arteriolar vasoconstriction. To further investigate this issue, we examined the effects of pinacidil, a K<sub>ATP</sub>-channel opener, on pressure-induced afferent arteriolar vasoconstriction. As shown in Fig. 5, pinacidil produced a concentration-dependent inhibition of myogenic reactivity, completely abolishing the response at 1.0 µM. These effects were totally reversed by glibenclamide. We had previously determined that pinacidil also inhibits angiotensin II-induced vasoconstriction and that these actions are also completely reversed by glibenclamide (37). The effects of pinacidil on myogenic vs. angiotensin II-induced vasoconstriction are compared in Fig. 6. Note that similar levels of afferent arteriolar vasoconstriction to elevated pressure and 0.1 nM angiotensin II were observed in these studies (Fig. 6B). As depicted in Fig. 6A, 1.0 µM pinacidil inhibited pressure-induced vasoconstriction by 97 ± 5% but inhibited the vasoconstrictor response to angiotensin II by only 59 ± 13% (P = 0.034). At 10 µM pinacidil, angiotensin II responses were inhibited by 84 ± 10%.

**DISCUSSION**

The present study demonstrates that the renal microvascular actions of CGRP involve both K<sub>ATP</sub>-dependent and K<sub>ATP</sub>-independent mechanisms. We found the efficacy of CGRP and the involvement of K<sub>ATP</sub> in its actions differ during myogenic vs. angiotensin II-induced afferent arteriolar vasoconstriction. Specifically, CGRP was more efficacious in inhibiting myogenic vasoconstriction, and this difference in efficacy was abolished by the K<sub>ATP</sub> channel blocker glibenclamide. These observations indicate that the afferent arteriolar actions of CGRP depend, in part, on the activation of K<sub>ATP</sub> during...
pressure-induced vasoconstriction but not during angiotensin II-induced vasoconstriction.

CGRP-induced renal vasodilation has been documented in numerous in vivo studies (reviewed in Ref. 39). Intravenous administration of CGRP increases both renal blood flow (2, 38) and glomerular filtration rate (2). High concentrations of CGRP induce severe hypotension and stimulate renin secretion, leading to an indirect reduction in renal blood flow (14, 15, 38). In the isolated perfused rat kidney, CGRP inhibits the vasoconstrictor actions of angiotensin II (23), norepinephrine (5, 16), and phenylephrine (6, 19). Edwards and Trizna (8) directly examined the actions of CGRP in isolated rabbit renal arterioles. These authors demonstrated that CGRP reversed norepinephrine-induced afferent arteriolar vasoconstriction but failed to elicit a significant vasodilation in efferent arterioles preconstricted with either norepinephrine or angiotensin II. Angiotensin II does not constrict efferent arterioles in this preparation (7). In the present study, we observed CGRP to exert more prominent vasodilatory actions on the afferent arteriole but to also significantly attenuate angiotensin II-induced efferent arteriolar vasoconstriction. Whether these differences regarding the efferent arteriolar actions of CGRP are attributed to species differences or are related to differences in the two experimental models cannot be ascertained at this time.

Our findings indicate that multiple mechanisms, including activation of $K_{ATP}$, contribute to the renal microvascular actions of CGRP. The CGRP$_1$ receptor subtype appears to mediate the vasodilatory actions of CGRP (5, 6, 11, 19). However, the signal transduction events following receptor activation are not completely resolved (3). Further complications arise from the possibility that the mechanisms of CGRP may vary among species and among vascular beds within the same species (33). The actions of CGRP in small renal arteries (<200 µm) are not affected by removal of the endothelium (13), and CGRP-induced renal vasodilation is reported to be either unaffected (15) or inhibited (2, 10) by $\text{N}^\circ\text{G}-\text{nitro-L-arginine methyl ester}$. Recent findings complicate interpretations of studies involving nitric oxide synthase (NOS) blockade, as basal NO and/or cGMP are known to play permissive roles in endothelium-independent responses, and NOS inhibition clearly affects vasodilatory responses that are not directly dependent on endothelial-derived NO (EDNO) (12). Although the present study did not directly address the role of the endothelium in CGRP-induced vasodilation, our findings indirectly suggest that mechanisms other than NO contribute to the actions of CGRP, as pressure-induced afferent arteriolar vasoconstriction in this model is not directly inhibited by EDNO (18) or other cGMP-dependent vasodilators (17).

The direct vasodilatory actions of CGRP have been linked to adenylate cyclase (3), and CGRP increases cAMP in glomeruli (8, 9) and mesangial cells (23). Quayle et al. (34) suggest that CGRP activates $K_{ATP}$ via cAMP-dependent protein kinase in rabbit mesenteric arteries. Nelson et al. (31) demonstrated that glibenclamide prevents CGRP-induced hyperpolarization in this preparation but only partially blocks the vasodilatory response. These authors also demonstrated that CGRP dilates vessels preconstricted with 50 mM K$^+$, suggesting K channel-dependent and -independent mechanisms. Gao et al. (13) found glibenclamide to have no effect on CGRP-induced vasodilations in renal arterial rings preconstricted with norepinephrine. Similarly, the hypotensive effects of intravenously administered CGRP in conscious rats are not affected by glibenclamide (1). Finally, Castellucci et al. (5) observed CGRP to reverse the vasoconstriction elicited by 60 mM KCl in the isolated perfused rat kidney, further suggesting that CGRP can elicit renal vasodilation through mechanisms independent of altered K conductance. In agreement with the above findings, glibenclamide failed to affect the vasodilatory actions of CGRP on angiotensin II-induced vasoconstriction in the present study. However, we found that the actions of CGRP on pressure-induced vasoconstriction were partially attenuated by blockade of $K_{ATP}$.

To our knowledge, the present study is the first to suggest that the nature of the underlying tone influences the mechanisms involved in the vasodilatory actions of CGRP. Thus $K_{ATP}$ appears to contribute to CGRP-induced vasodilation during pressure-induced but not during angiotensin II-induced vasoconstriction. Our finding that the $K_{ATP}$ opener pinacidil is more potent against myogenic vs. angiotensin II-dependent tone also supports the concept that the ability of $K_{ATP}$ to contribute to vasodilatory responses is dependent on the vasoconstrictor stimulus. It should be mentioned that the pattern of response to CGRP and pinacidil during myogenic vs. angiotensin II-induced vasoconstriction is not typical of that observed with other vasodilators. For example, atrial natriuretic peptide readily reverses afferent arteriolar vasoconstriction induced by agonists, including angiotensin II (24, 27), but has little effect on myogenic vasoconstriction (17). A similar pattern is seen with the NO-dependent component of acetylcholine-induced vasodilation (18). In contrast, calcium antagonists are equally efficacious in inhibiting both types of vasoconstriction (26).

We consider at least two possible explanations for the differing involvement of $K_{ATP}$ in the vasodilatory actions of CGRP in these two settings. First, it is possible that differences in membrane potentials could contribute to the differing sensitivities of pressure- and angiotensin II-induced vasoconstriction to K channel-induced hyperpolarization. Although both stimuli evoke afferent arteriolar vasoconstriction by activating L-type Ca channels (for review, see Ref. 26), the responses could be associated with differing degrees of depolarization and, accordingly, exhibit differing sensitivities to submaximal levels of K channel activation. It should be noted, however, that in our studies we were careful to examine angiotensin II- and pressure-induced responses at comparable levels of vasoconstriction (Figs. 4B and 6B). Alternatively, it is possible that the activation of $K_{ATP}$ is directly affected by the vasoconstrictor signaling
pathways. In a series of studies using cultured porcine coronary artery myocytes, Miyoshi and co-workers found extracellular application of angiotensin II (29), vasopressin (40), and endothelin (30) to inhibit a K channel that, like K_{ATP}, was inhibited by intracellular ATP and activated by nicorandil. Similarly, Kleppisch and Nelson (20) demonstrated using freshly isolated cerebral artery myocytes that 5-hydroxytryptamine and histamine inhibit a pinacidil-induced, glibenclamide-sensitive K current. These actions were mimicked by phorbol ester, suggesting that protein kinase C may inhibit K_{ATP} (20). Both groups have proposed that inhibition of K_{ATP} contributes to agonist-induced depolarization and vasoconstriction. However, blockade of K_{ATP} by glibenclamide has no effect on either basal tone or angiotensin II-induced vasoconstriction in the afferent or efferent arteriole (Ref. 37, Fig. 4). Thus our data do not support a role of agonist-induced inhibition of K_{ATP} in the vasoconstrictor response of renal arterioles to angiotensin II. Nevertheless, this mechanism may modulate the actions of vasodilators. Thus angiotensin II-induced inhibition of K_{ATP} may account for the differing potencies of pinacidil and CGRP on angiotensin II- vs. pressure-induced vasoconstriction and for the differing involvement of K_{ATP} in the actions of CGRP in these two settings.

In conclusion, the present study demonstrates that CGRP attenuates the afferent and efferent arteriolar actions of angiotensin II but is more potent in inhibiting the myogenic response of the afferent arteriole. Multiple mechanisms including activation of K_{ATP} appear to contribute to the vasodilatory actions of CGRP during pressure-induced afferent arteriolar vasoconstriction. However, K_{ATP} does not appear to contribute to the afferent or efferent arteriolar actions of CGRP during angiotensin II-induced vasoconstriction. The manner by which the nature of the underlying vasoconstrictor tone influences the vasodilatory mechanisms of CGRP is not currently known. We suggest that angiotensin II-induced inhibition of K_{ATP} contributes to this phenomenon.

We are indebted to Qing Fei for expert technical assistance with some of these studies.

This study was supported by grants from the Medical Research Council of Canada, the Alberta Heart and Stroke Foundation, and the Kidney Foundation of Canada. M. Reslerova was supported by a Fellowship Award from the Kidney Foundation of Canada. R. Loutzenhiser is a Medical Scholar of the Alberta Heritage Foundation for Medical Research. M. Reslerova was supported by a Fellowship Award from the Kidney Foundation of Canada. R. Loutzenhiser is a Medical Scholar of the Alberta Heritage Foundation for Medical Research.

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Received 19 November 1997; accepted in final form 18 February 1998.

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