Mechanism of Action of Angiotensin II and Bradykinin on Prostaglandin Synthesis and Vascular Tone in the Isolated Rat Kidney

Effect of Ca++ Antagonists and Calmodulin Inhibitors

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SUMMARY. We have studied the effect of angiotensin II and bradykinin on prostaglandin output and vascular tone during extracellular calcium depletion and administration of calcium antagonists and calmodulin inhibitors to elucidate the mechanism of action in the isolated rat kidney perfused with Tyrode's solution. Administration of angiotensin II (0.028-0.28 nmol) or bradykinin (0.28-2.8 nmol) enhanced the output of prostaglandin E2 and 6-keto-prostaglandin F1α in a dose-dependent manner. Angiotensin II, but not bradykinin, produced renal vasoconstriction. Omission of calcium from the medium or infusion of calcium entry blockers, diltiazem (60 μM), or nimodipine (47 μM), failed to alter prostaglandin output elicited by angiotensin II or bradykinin; however, the effect of angiotensin II to produce renal vasoconstriction was inhibited. If calcium was omitted from the medium, the intracellular calcium antagonists, 8-(diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride (23 μM), dantrolene sodium (31 μM), or ryanodine (2 μM), attenuated prostaglandin output caused by angiotensin II but not bradykinin. Calmodulin inhibitors, trifluoperazine (2 μM), naphthalene sulfonamide hydrochloride (2 μM), or calmidazolium (2 μM), diminished prostaglandin output elicited by angiotensin II, but not that caused by bradykinin. Trifluoperazine, but not naphthalene sulfonamide or calmidazolium, attenuated the renal vasoconstrictor effect of angiotensin II. Prostaglandin output induced by angiotensin II and bradykinin were inhibited by mepacrine and indomethacin, whereas, the prostaglandin output caused by exogenous arachidonic acid (33 nmol) was abolished by indomethacin but was unaltered by mepacrine, calcium antagonists, and calmodulin inhibitors. From these data, we conclude that angiotensin II produces renal vasoconstriction by a mechanism dependent on extracellular calcium but not calmodulin, whereas angiotensin II-induced prostaglandin output depends on intracellular calcium and calmodulin. In contrast, bradykinin appears to stimulate prostaglandin synthesis by a calcium/calmodulin-independent mechanism. (Circ Res 56: 97-108, 1985)
cular Ca++ antagonists, dantrolene sodium (Morgan and Bryant, 1977), ryanodine (Sutko and Kenyon, 1983), and TMB-8∗ (Chiu and Malagodi, 1975), on the AII and BK action on prostaglandin output and vascular tone in the isolated rat kidney perfused with or without Ca++-containing Tyrode’s solution. Since several biological actions of Ca++ are mediated by its interaction with calmodulin, we have investigated the effect of three structurally distinct calmodulin inhibitors, trifluoperazine (Levin and Weiss, 1976), W-7∗ (Hidaka et al., 1981), and calmidazolium (Van Belle, 1981), to determine the possible contribution of this Ca++-binding protein to the actions of AII and BK on prostaglandin synthesis and vascular tone.

Methods

Male Sprague-Dawley rats (weighing 350–400 g), maintained on regular Purina chow diet, were anesthetized with ether, and the abdomen was opened by a midline laparotomy. The aorta was ligated proximal and distal to the left renal artery, and a catheter was inserted into the renal artery. The kidney was flushed with heparinized saline (100 U/ml), isolated from the animal, and perfused with Tyrode’s solution at a constant flow rate of 5 ml/min, using a Harvard peristaltic pump as described previously (Malik and Wiffing, 1975). The perfusion fluid was maintained at 37°C and gassed with a mixture of 95% O2 and 5% CO2. The renal perfusate was allowed to flow out of the cut ends of the renal vein and ureter, and was not recirculated. The composition of Tyrode’s solution in mmol/liter was as follows: NaCl, 137; KCl, 2.7; CaCl2, 1.8; MgCl2, 1.1; NaHCO3, 12; NaH2PO4, 0.42; and D(+)-glucose, 5.6. Perfusion pressure was measured with a pressure transducer (Statham P23 ID) and recorded on a physiograph (Esterline Angus). Since the flow rate was maintained constant, changes in perfusion pressure reflect alterations in renal vascular resistance.

Experimental Protocol

The following eight series of experiments were performed.

Series 1

The purpose of this series of experiments was to investigate the effects of AII, BK, and the Ca++ ionophore, A-23187, on the renal output of PGE2 and 6-keto-PGF1α, and on vascular tone in kidneys perfused with and without added Ca++ in the absence or presence of EGTA (23 μM). In kidneys perfused with Tyrode’s solution containing normal (1.8 mM), lower (0.6 mM), or higher (5.4 mM) Ca++, drugs were administered during 35–125 minutes of perfusion, whereas in kidneys perfused with Ca++-free Tyrode’s solution, the drugs were given during 80–155 minutes of perfusion, since the basal output of prostaglandins was stable during these periods. Moreover, the basal output of prostaglandins and the perfusion pressure during these periods were also similar in kidneys perfused with or without Ca++-containing Tyrode’s solution. AII (0.028–0.28 nmol), BK (0.28–2.8 nmol), A-23187 (9.6 nmol), or their respective vehicles, were injected as a bolus at 15-minute intervals in a random order into the side port of the tubing leading to the renal artery. Changes in perfusion pressure were recorded and renal perfusate samples were collected for 2-minute periods just prior to and after the injections of AII, BK, A-23187, or their vehicles. The samples were frozen at −20°C for later analysis of prostaglandins.

Series 2

The effect of AII and BK on the renal output of prostaglandins and on vascular tone was examined in the presence of the Ca++ channel blockers, diltiazem (60 and 300 μM), nimodipine (47 and 235 μM), or their respective vehicles. After a 35-minute stabilization period, AII (0.097 nmol), BK (0.94 nmol), or their vehicles were injected at 15-minute intervals in a random order. Changes in perfusion pressures were recorded, and samples of the renal perfusate were collected for prostaglandin determination as described for series 1 experiments.

Series 3

This series of experiments was performed to investigate the effect of the intracellular Ca++ antagonists, dantrolene sodium, TMB-8, and ryanodine on the actions of AII and BK on prostaglandin synthesis and vascular tone in kidneys perfused with Tyrode’s solution in the absence and presence of 1.8 mM Ca++. Dantrolene sodium (31 μM), TMB-8 (2.3 μM), or ryanodine (2 μM) was added to the perfusion medium, and, after 80 minutes of stabilization, AII (0.097 nmol), BK (0.94 nmol), or their vehicles were injected into the kidney in a random order at 15-minute intervals. In an additional series of experiments, the kidneys were perfused with Tyrode’s solution containing 0.9 or 0.6 mM Ca++ and TMB-8 (2.3 μM), and the effect of AII (0.097 nmol) on prostaglandin output and on renal vascular tone was determined.

Series 4

The effect of trifluoperazine, W-7, and calmidazolium was studied in concentrations reported to inhibit calmodulin activity (Levin and Weiss, 1976; Hidaka et al., 1981; Van Belle, 1981), on AII- and BK-induced prostaglandin output and on their vascular actions in the absence and presence of 1.8 mM Ca++. Trifluoperazine (2 μM), W-7 (2 μM), calmidazolium (0.2 μM), or their vehicles were added to the perfusion fluid, and AII (0.097 nmol), BK (0.94 nmol), or their vehicles were injected into the renal arterial catheter at 15-minute intervals.

Series 5

The purpose of these experiments was to study the effect of the AII receptor antagonist [Sar1,Ala8]-AII on AII and BK actions on prostaglandin output and vascular tone in kidneys perfused without and with Ca++-containing Tyrode’s solution. [Sar1,Ala8]-AII (0.02 or 1.1 μM) was added to the perfusion medium. AII (0.097 nmol), BK (0.94 nmol), or their vehicles were injected at 15-minute intervals, and the perfusate was collected as described for series 1 experiments.

Series 6

The purpose of this series of experiments was to examine the effect of Ca++ antagonists, calmodulin inhibitors, and [Sar1,Ala8]-AII on arachidonic acid-induced prostaglandin output and alterations in vascular tone in kid-

* TMB-8, 8-(diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride; W-7, naphthylenedisulfonamide hydrochloride.
nerys perfused with or without Ca**+-containing Tyrode’s solution. Diltiazem (60 μM), nimodipine (47 μM), dantrolene sodium (31 μM), TMB-8 (2.3 μM), ryanodine (2 μM), trifluoperazine (2 μM), W-7 (2 μM), calmidazolium (0.2 μM), or their vehicles were added to the perfusion medium, and arachidonic acid (33 nmol), or its vehicle was injected into the kidney.

**Series 7**

This series of experiments was performed to determine the effect of a combined injection of All and BK on prostaglandin output and on vascular tone in kidneys perfused with Ca**+-containing Tyrode’s solution. All (0.097 nmol) plus BK (0.94 nmol) or their vehicles were injected, and the renal perfusate samples were collected, by a protocol similar to that described in series 1.

**Series 8**

The effect of indomethacin and mepacrine on vascular tone and prostaglandin output elicited by All, BK, and arachidonic acid was determined in the presence or absence of Ca**+ (1.8 μM). The kidney was perfused with Tyrode’s solution containing indomethacin (2.8 μM), mepacrine (21 μM), or their vehicles with or without Ca**+. All (0.097 nmol), BK (0.94 nmol), arachidonic acid (33 nmol), or their vehicles were injected into the renal arterial catheter by a protocol similar to that described for series 1 experiments.

**Determination of Prostaglandins**

Prostaglandin E2 and 6-keto-prostaglandin F1α (the stable hydrolysis product of PGI2) in the renal perfusate were measured by radioimmunoassay as described previously (Shaffer and Malik, 1982). The limit of detection of PG in our assay was: 2 pg for PGE2, and 5 pg for 6-keto-PGF1α. The cross-reactivity for each antibody was less than 0.5% for other prostaglandins and their metabolites. Extraction, was 90 to 95%, and none of the drugs used in our experiments interfered with the radioimmunoassay.

**Drugs**

The following drugs used in this study were purchased: bradykinin diacetate (Protein Research Foundation, Minoh-Shi, angiotensin II (Beckman Instruments, Inc.), indomethacin, quinacrine dihydrochloride (mepacrine), and ethylenglycol-bis-(β-amin-ethyl ether) N',N'-tetraacetic acid (EGTA, Sigma Chemical Co.), arachidonic acid (Nu-Cher Prep Inc.), 8-(diethylamino) octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8, Aldrich Chemical Co.), naphthalene-sulfonamide hydrochloride (W-7, Rikaken Co.), calmidazolium (Janssen Pharmaceutical) and [Sar1,Ala8]-AII was dissolved in 0.15 M NaCl and mepacrine in polyethylene glycol 400 (2.5 mg/ml) and infused into the renal arterial circuit. Diltiazem, TMB-8, dantrolene sodium, ryanodine, trifluoperazine, W-7, calmidazolium, and mepacrine were dissolved in small volumes of Tyrode’s solution with or without Ca**+, depending upon the experimental protocol, and added to the perfusion fluid to obtain the final concentration.

**Analysis of Data**

The basal and the increase in prostaglandin output elicited by various agents is expressed as ng/min of immunoreactive PGE2 and 6-keto-PGF1α. The basal output represents the amount of prostaglandins in the samples collected for a 2-minute period immediately before administration of a drug. The increase in the output of prostaglandins caused by a drug was calculated by subtracting the basal efflux of prostaglandins from that obtained during 2-minute periods immediately after the injection of a drug. The results are expressed as mean ± SEM. The data were analyzed by one-way analysis of variance, and Dunnett’s test was applied to determine the difference between means and Student’s t-test for unequal sample sizes (Zar, 1984). Differences between means were considered significant if the probability (P) of the null hypothesis being true was less than 0.05.

**Results**

**Effect of All, BK, and A-23187 on the Output of Prostaglandins and Vascular Tone in the Rat Kidney Perfused with or without Ca**+ (1.8 mm)-Containing Tyrode’s Solution (Figs. 1 and 2)

The basal perfusion pressure remained at a steady level (56 ± 4 mm Hg, n = 6) for 3–4 hours in kidneys perfused with or without Ca**+-containing Tyrode’s solution. During this period of perfusion the wet weight of the kidney, which averaged 1.6 g (range 1.5–1.8 g), was unchanged. In kidneys perfused with Ca**+ (1.8 mm) -containing Tyrode’s solution, administration of All (0.028–0.28 nmol) into the renal arterial circuit increased perfusion pressure and the output of PGE2 and 6-keto-PGF1α. Injections of BK (0.28–2.8 nmol) into the kidney also increased the output of PGE2 and 6-keto-PGF1α, but had no effect on perfusion pressure. The rise in perfusion pressure elicited by All peaked in 30–40 seconds, whereas the output of prostaglandins caused by All and BK reached maximal levels in 2 minutes and gradually
FIGURE 1. Effect of AII on the output of PGE₂ (upper panel) and 6-keto-PGF₁α (middle panel) and on the perfusion pressure (bottom panel) in the isolated rat kidney perfused with and without Ca²⁺-containing Tyrode’s solution. • denotes values different from basal, whereas * represents the difference between the values obtained in the presence and absence of Ca²⁺. (P < 0.05).

FIGURE 2. Effect of BK on the output of PGE₂ (upper panel) and 6-keto-PGF₁α (middle panel) and on the perfusion pressure (bottom panel) in the isolated rat kidney perfused with and without Ca²⁺-containing Tyrode’s solution. • denotes values different from basal (P < 0.05).

With Ca²⁺-free Tyrode’s solution, injections of AII (0.028–0.28 nmol) or BK (0.28–2.8 nmol) did not alter the perfusion pressure, but enhanced the output of prostaglandins. Addition of Ca²⁺ (1.8 mM) to the perfusion medium restored the vasoconstrictor effect of AII to levels observed in kidneys perfused with normal Tyrode’s solution. Both AII and BK produced a greater increase in the output of PGE₂ than 6-keto-PGF₁α in the presence, as well as in the absence, of Ca²⁺. In kidneys perfused with Ca²⁺-free Tyrode’s solution containing EGTA, the basal output of prostaglandins (1.4 ± 0.3 ng/min PGE₂ and 1.2 ± 0.3 ng/min 6-keto-PGF₁α) or the rise in prostaglandin output elicited by AII (0.097 nmol) (3.4 ± 0.2 ng/min PGE₂ and 1.9 ± 0.2 ng/min 6-keto-PGF₁α) and by BK (0.94 nmol) (2.2 ± 0.3 ng/min PGE₂ and 1.3 ± 0.1 ng/min 6-keto-PGF₁α) (P < 0.05, n = 4) was the same as that observed in kidneys perfused with Ca²⁺-free Tyrode’s solution in the absence of EGTA (Figs. 1 and 2) (P > 0.05).

In kidneys perfused with Tyrode’s solution containing higher (5.4 mM) or lower (0.6 mM) Ca²⁺, the output of prostaglandins elicited by AII (0.097 nmol) or BK (0.94 nmol) was the same as that obtained in the presence of normal Ca²⁺ (data not shown). However, the rise in perfusion pressure elicited by AII was significantly higher in the presence of 5.4 mM Ca²⁺ (58 ± 9 mm Hg, n = 6) and lower in the presence of 0.6 mM Ca²⁺ (18 ± 3 mm Hg, n = 6) than that observed during normal Ca²⁺, 1.8 mM (32 ± 6 mm Hg, n = 6) (P < 0.05). The basal output of prostaglandins or the perfusion pressure were not altered by raising or lowering Ca²⁺ concentration in the perfusion medium.

Administration of A-23187 (9.6 nmol) into the kidney perfused with Ca²⁺ (1.8 mM) -containing Tyrode’s solution increased the output of PGE₂ and 6-keto-PGF₁α, from 0.99 ± 0.20 to 3.11 ± 0.51 ng/min and 1.03 ± 0.15 to 2.72 ± 0.4 ng/min, respectively (P < 0.05, n = 6), but failed to alter perfusion pressure. In the absence of Ca²⁺, A-23187 failed to alter either the output of prostaglandins or perfusion pressure. Injections of each drug vehicle into the renal arterial circuit did not alter the output of prostaglandins or the perfusion pressure in the presence or absence of Ca²⁺.
Effect of Diltiazem and Nimodipine on the Actions of All and BK on Prostaglandin Output and Vascular Tone (Figs. 3 and 4)

In kidneys perfused with Tyrode's solution containing diltiazem or nimodipine, administration of All (0.097 nmol) increased the renal output of PGE$_2$ and 6-keto-PGF$_{1 \alpha}$. The magnitude of this increase was not different from that obtained in the presence of the vehicles of these agents. The rise in perfusion pressure produced by All was significantly attenuated during infusion of diltiazem or nimodipine (Fig. 3). The output of prostaglandins elicited by BK (0.94 nmol) was not altered by the Ca$^{++}$ channel blockers (Fig. 4). During infusion of diltiazem or nimodipine, the basal output of prostaglandins or perfusion pressure was not altered. Increasing the concentration of the Ca$^{++}$ channel blockers in the perfusion medium by 5-fold also failed to alter the rise in PG output elicited by All or BK. In the presence of 5-fold higher concentrations of diltiazem or nimodipine, All still significantly increased perfusion pressure from 54 ± 3 to 69 ± 2 mm Hg and from 55 ± 2 to 64 ± 3 mm Hg, respectively (P < 0.05, n = 7 and n = 5, respectively).

Effect of Dantrolene Sodium, TMB-8, or Ryanodine on All and BK Actions on Prostaglandin Output and Vascular Tone in the Absence and Presence of Ca$^{++}$ (Figs. 5 and 6).

In kidneys perfused with Ca$^{++}$-free Tyrode's solution containing dantrolene sodium, TMB-8, or ryanodine, the effect of All (0.097 nmol), but not that of bradykinin (0.94 nmol), to enhance the output of PGE$_2$ and 6-keto-PGF$_{1 \alpha}$ was significantly reduced. Addition of Ca$^{++}$ (1.8 mM) to the medium abolished the inhibitory effect of intracellular Ca$^{++}$ antagonists on All-induced prostaglandin output and renal vasoconstriction. In kidneys perfused with Tyrode's solution containing normal Ca$^{++}$ (1.8 mM), the intracellular Ca$^{++}$ antagonists also failed to alter All- or BK-induced output of prostaglandins and the rise in perfusion pressure caused by All. However, in kidneys perfused with solution containing 0.9 mM Ca$^{++}$ and TMB-8, the output of prostaglandins was reduced, whereas the rise in perfusion pressure elicited by All remained unaltered (Fig. 6). In the presence of 0.6 mM Ca$^{++}$ addition of TMB-8 reduced both the prostaglandin output and the rise in perfusion pressure caused by All (Fig. 6). The basal output of prostaglandins and the perfusion pressure were unaltered by variations in Ca$^{++}$ concentrations and/or addition of intracellular Ca$^{++}$ antagonists to the medium.
Effect of Trifluoperazine, W-7, and Calmidazolium on the Actions of All and BK on Prostaglandin Output and Vascular Tone in the Presence or Absence of Ca++ (Figs. 7 and 8)

Infusion of trifluoperazine, W-7, or calmidazolium in the presence of Ca++ inhibited the output of both PGE₂ and 6-keto-PGF₁α elicited by All. Trifluoperazine, but not W-7 or calmidazolium, attenuated the rise in perfusion pressure elicited by All (0.097 nmol). In the absence of Ca++, the calmodulin inhibitors also attenuated the rise in prostaglandin output produced by All. The effect of BK (0.94 nmol) to increase the output of prostaglandins was unaltered by the calmodulin inhibitors in the presence or absence of Ca++. The basal output of prostaglandins or perfusion pressure was also unaffected by the calmodulin inhibitors.

Effect of [Sar¹,Ala⁸]-AII on the Actions of All and BK on Prostaglandin Output and Vascular Tone in the Presence or Absence of Ca++ (Fig. 9)

In kidneys perfused with Ca++-containing (1.8 mM) or Ca++-free Tyrode’s solution, infusion of [Sar¹,Ala⁸]-AII reduced the effect of All (0.097 nmol) to increase prostaglandin output and the perfusion pressure; the magnitude of decrease was the same in the absence as in the presence of Ca++. Infusion of higher concentration of [Sar¹,Ala⁸]-AII abolished both the output of prostaglandins and renal vasorelaxation produced by All; the prostaglandin output elicited by BK (0.94 nmol) was not altered by either the low or high concentration of the All receptor antagonist (data not shown). [Sar¹,Ala⁸]-AII did not alter the basal output of prostaglandins or perfusion pressure in the presence or absence of Ca++.
Effect of Ca++ Depletion, Ca++ Antagonists, Calmodulin Inhibitors, and [Sar1,Ala8]-AII on the Actions of Arachidonic Acid on Prostaglandin Output and Vascular Tone

Administration of arachidonic acid (33 nmol) enhanced the output of PGE2 and 6-keto-PGF1α from 1.42 ± 0.24 to 6.91 ± 0.80 ng/min and from 1.32 ± 0.11 to 6.21 ± 0.40 ng/min, respectively, and enhanced the perfusion pressure from 57 ± 4 to 69 ± 2 mmHg (P < 0.05, n = 5) in kidneys perfused with Ca++-containing Tyrode's solution. In kidneys perfused with Ca++-depleted solution, administration of arachidonic acid produced similar increases in the output of prostaglandins, but it failed to increase perfusion pressure. The effect of arachidonic acid to enhance prostaglandin synthesis was not altered by diltiazem, nimodipine, dantrolene sodium, TMB-8, ryanodine, trifluoperazine, W-7, calmidazolium or [Sar1,Ala8]-AII. The increase in perfusion pressure elicited by arachidonic acid in the presence of Ca++...
was reduced by diltiazem (60 μM) from 18 ± 6 mmHg (n = 6) to 8 ± 1 mmHg (n = 6) (P < 0.05) and by nimodipine (47 μM) from 18 ± 6 mmHg (n = 6) to 13 ± 5 mmHg (n = 7) (P < 0.05) but not by other agents.

**Effect of All plus BK on the Output of Prostaglandins and Vascular Tone in the Presence of Ca++ (Table 1)**

Administration of the combination of All and BK into kidneys perfused with normal Tyrode's solution enhanced the output of PGE₂ and 6-keto-PGF₁α. Prostaglandin output elicited by this combination was significantly greater than that elicited by the separate administration of All or BK. The rise in perfusion pressure elicited by All plus BK was not significantly different from that elicited by the injection of All alone.

**Effect of Indomethacin and Mepacrine on the Actions of All, BK, and Arachidonic Acid on Prostaglandin Output and Vascular Tone in the Presence or Absence of Ca++ (Fig. 10)**

During infusion of indomethacin, in the presence of Ca++, All (0.097 nmol), BK (0.94 nmol), or arachidonic acid (33 nmol)-induced prostaglandin output was completely prevented. Infusion of mepacrine abolished All and BK but not arachidonic acid-induced output of PGE₂ and 6-keto-PGF₁α. The basal prostaglandin output was reduced by indomethacin but not by mepacrine. The effect of indomethacin and mepacrine on prostaglandin output elicited by All, BK, or arachidonic acid in the absence of Ca++ was similar as in the presence of Ca++ (data not shown). The increase in perfusion pressure elicited by All or arachidonic acid in the presence of Ca++ was reduced during infusion of indomethacin or mepacrine (Fig. 10).

**Discussion**

The present study in the isolated rat kidney perfused with Tyrode's solution indicates that All requires extracellular Ca++ to produce renal vasoconstriction and intracellular Ca++ to stimulate prostaglandin synthesis. Only the stimulation of prostaglandin synthesis depends upon calmodulin, a Ca++-binding protein (Cheung, 1980). In contrast, BK stimulates prostaglandin synthesis by a mechanism which appears to be independent of both extra- and intracellular Ca++ and calmodulin.

**All-Induced Vasoconstriction**

In the isolated rat kidney perfused with Tyrode's solution containing Ca+++, administration of All produced renal vasoconstriction and increased the output of PGE₂ and 6-keto-PGF₁α, in a dose-related
manner, a finding consistent with that reported in other species (Needleman et al., 1973; McGiff, 1981). Removal of Ca** from the perfusion medium abolished All-induced renal vasoconstriction but failed to alter prostaglandin output elicited by All which suggests a selective requirement of extracellular Ca** in the former but not in the latter action of the peptide. Loss of All-induced renal vasoconstriction was not due to disruption of vascular smooth muscle contractile elements, since addition of Ca** to the perfusion fluid restored the vasoconstrictor effect of All. The selective involvement of extracellular Ca** to the renal vasoconstriction and not to prostaglandin output elicited by All was also suggested by our findings that (1) raising or lowering Ca** concentrations in the perfusion medium augmented or reduced, respectively, the renal vasoconstriction but not the output of prostaglandins produced by All and (2) two structurally dissimilar Ca** antagonists, diltiazem, and nimodipine (Towart and Kazda, 1979; van Breeman et al., 1981; Towart, 1981), attenuated All-induced renal vasoconstriction without altering its effect on prostaglandin output. These observations, taken together with the demonstration that All increases uptake of 45Ca into the vascular smooth muscle (Deth and van Breeman, 1974), suggest that All produces renal vasoconstriction primarily by increasing the influx of extracellular Ca**, a finding which is consistent with those reported in the rabbit aorta (Deth and van Breeman, 1974; St. Louis et al., 1977).

Our finding that [Ser1,Ala8]-All, a selective All receptor antagonist, abolished the renal vasoconstrictor effect of All but not that of a structurally dissimilar peptide, arginine vasopressin (unpublished observation), suggests that All increases the influx of Ca** consequent to its interaction with a specific receptor. The interaction of All with its receptor may enhance Ca** influx by a mechanism coupled with membrane depolarization (voltage-operated channels) and/or by a nonelectrical mechanism (receptor-operated channels). Our demonstration that diltiazem and nimodipine, Ca** entry blockers (Towart and Kazda, 1979; van Breeman et al., 1981; Towart, 1981), like nifedipine (Jover et al., 1982), attenuated All-induced renal vasoconstriction suggests that All could activate voltage-operated channels (Freer et al., 1976; Mironneau and Gargouil, 1979; Kass and Blair, 1981). Nevertheless, our observation that removal of Ca** from the perfusion fluid was more effective than the Ca** entry blockers in inhibiting the renal vasoconstrictor effect of All suggests that the peptide is unlikely to increase Ca** influx only through voltage-sensitive channels.

Enhanced influx of Ca** through receptor- or voltage-operated channels consequent to All-receptor interaction may release intracellularly bound Ca** (Ford and Podolshky, 1972) or act directly to activate vascular smooth muscle contractile elements. In the present study, dantrolene sodium, ryanodine, or TMB-8—agents that inhibit release of Ca** from intracellular sites (Chiou and Malagodi, 1975; Morgan and Bryant, 1977; Sutko and Kenyon, 1983)—failed to alter All-induced renal vasoconstriction in the presence of extracellular Ca** (1.8 mm), suggesting that intracellular Ca** is not utilized in this action of the peptide. However, we cannot exclude a small contribution of intracellular Ca** in the vasoconstriction elicited by All, since, in the presence of low Ca** (0.6 mm), TMB-8 attenuated All-induced renal vasoconstriction.

Ca** in the vascular smooth muscle may interact with calmodulin to increase the activity of myosin kinase which phosphorylates the light chain of myosin and allows the latter to interact with actin to initiate vascular smooth muscle contraction (Adelstein et al., 1982). Supporting this proposal is the demonstration that All stimulates and chlorpromazine, a calmodulin inhibitor, attenuates myosin light chain phosphorylation in cultured vascular smooth muscle cells (Anderson et al., 1981). Moreover, agents that inhibit Ca**-calmodulin-dependent myosin kinase reduce the contraction of rabbit aorta produced by All (Asano et al., 1982). However, much higher concentrations of the calmodulin inhibitors were required to reduce contraction of the vascular smooth muscle produced by All than those caused by other agonists (Peach, 1981; Asano et al., 1982). In the present study, trifluoperazine attenuated All-induced renal vasoconstriction; however, two structurally dissimilar and relatively more specific calmodulin inhibitors, W-7 (Hidaka et al., 1981) and calmidazolium (Van Belle, 1981), failed to alter the renal vasoconstrictor effect of this peptide. Thus, it appears that the All-induced rise in cellular Ca** concentration could increase myosin kinase activity and produce renal vasoconstriction by a mechanism unrelated to calmodulin.

**BK Actions on Vascular Tone**

Bradykinin, which is known to produce renal vasodilation in vivo in several species, and relaxation, contraction, or no effect in the isolated perfused vessels or arterial strips (Regoli and Barabé, 1980), enhanced the prostaglandin output but did not alter the renal vascular tone in our preparation in the presence of Ca**. The inability of bradykinin to produce alterations in vascular tone in the present study could be due to loss of intrinsic tone in the in vitro Tyrode-perfused kidney. However, this is unlikely, since histamine, a renal vasodilator, at doses of 2.4, 4.8, and 24 nmol, reduced the perfusion pressure by 10, 18, and 28 mmHg, respectively (unpublished observation). Whether bradykinin requires the presence of some blood-borne factors for expression of its vasodilator action in the kidney in vitro is unknown.

**All- and BK-Induced Prostaglandin Output**

An important finding in the present study was that depletion of Ca** from the perfusion medium...
in the absence or presence of EGTA or addition of Ca++ entry blockers, which attenuated All-induced renal vasoconstriction, did not alter the effect of All and BK to enhance the output of prostaglandins. These findings, and our observation that a Ca++ ionophore, A23187, which increased the output of PGE2 and 6-keto-PGF1a, without altering the vascular tone, failed to alter prostaglandin output in the absence of Ca++, suggest that (1) the effect of All to increase renal output of prostaglandins is not the result of alterations in vascular tone, and (2) both All and BK stimulate renal prostaglandin output by a mechanism that does not require extracellular Ca++. Juan (1979) has reported that, in the rabbit ear artery, the effect of bradykinin to stimulate the output of PGE2 was not altered by removal of Ca++ from the medium and was reduced only by adding high amounts of EGTA to the Ca++-depleted medium. In the rabbit portal vein, in the absence of Ca++ and in the presence of EGTA, the output of PGE2 elicited by All was not completely prevented (Simmet and Heritger, 1980). However, in the bovine pulmonary artery (Crutchley, 1983) and porcine aortic endothelial cells (Whorton et al., 1982), renal medullary slices (Benabé et al., 1982; Zenser et al., 1982), or glomerular mesangial cells in culture (Scharschmidt and Dunn, 1983), the effect of All or BK to stimulate prostaglandin synthesis was abolished by the lack of Ca++ or by addition of Ca++ channel blockers in the medium. Loss of the effect of All or BK to stimulate prostaglandin synthesis in these tissues, but not in the intact perfused kidney in the absence of Ca++, could be due to differences in experimental conditions (incubation vs. perfusion and/or time of preincubation without Ca+++) and/or the response of various renal cellular structures (vascular and glomerular mesangium, medullary interstitium, cortical and medullary tubules) to the peptides. In spite of differences in the response of renal medullary slices or mesangial cells in culture and perfused kidney to All or BK during removal of Ca++ for the medium, the profile of prostaglandins released by the peptides in the former tissues was similar to that obtained in the intact kidney.

Prostaglandin synthesis elicited by All and BK in the rat kidney, as in the rabbit kidney (Needleman et al., 1973), was due to activation of two distinct types of receptors because [Sar1,Ala8]-All, a selective All receptor antagonist, inhibited the output of prostaglandins elicited by All but not BK. Although the binding of All with its receptor sites has been reported to be influenced by divalent cations (Wright et al., 1982), Ca++ in our study did not appear to alter the action of All on its receptor to increase prostaglandin output for the following reasons. First, the effect of All to increase the output of PGE2 or 6-keto-PGF1a was not affected by changes in Ca++ concentration or removal of Ca++ from the medium. Second, the magnitude of inhibition of All-induced prostaglandin output by [Sar1,Ala8]-All, in the absence of Ca++ was similar to that observed in the presence of Ca++.

Prostaglandin synthesis elicited by All and BK in the absence of extracellular Ca++ could be due to the mobilization of Ca++ from the cell membrane or intracellular structures, which may in turn cause activation of phospholipase A2 or other lipases that release arachidonic acid from tissue phospholipids for prostaglandin synthesis. Our demonstration that an intracellular Ca++ antagonist, TMB-8 [which reduces thrombin and A23187-induced arachidonic acid or prostaglandin release from platelets (Rittenhouse-Simmons and Deykin, 1978; Brotherton and Hoak, 1982) and other intracellular Ca++ antagonists, dantrolene sodium and ryanodine (Chiou and Malagodi, 1975; Morgan and Bryant, 1977), abolished prostaglandin output elicited by All, but not BK, suggests that the former but not the latter peptide acts by a mechanism requiring intracellular Ca++. The possibility that BK stimulates prostaglandin synthesis by mobilizing Ca++ from intracellular sites that are resistant to blockade by the above intracellular Ca++ antagonists cannot be totally excluded. Alternatively, it is possible that BK promotes prostaglandin synthesis by activating a lipase that is distinct from that involved in the action of All. That All stimulates prostaglandin synthesis by a mechanism involving mobilization of intracellular Ca++ is also suggested by the demonstrated ability of this peptide to release Ca++ from microsomes in the aorta (Baudouin et al., 1972). The inhibitory action of the intracellular Ca++ antagonists on All-induced prostaglandin synthesis in our study was not due to the action of these agents to interfere with the action of All at its receptor, or due to a reduction in cyclooxygenase activity since: (1) in kidneys perfused with Tyrode's solution containing 0.9 mM Ca++, the intracellular Ca++ inhibitor, TMB-8, attenuated the output of prostaglandins without altering the renal vasoconstriction elicited by All, and (2) the output of PGE2 and 6-keto-PGF1a elicited by arachidonic acid was not altered by the intracellular Ca++ antagonists. These findings, together with our demonstration that increasing or decreasing the Ca++ concentration in the perfusion medium enhanced or reduced the renal vasoconstriction, respectively, without altering the output of prostaglandins elicited by All, suggest that it is improbable that the extracellular Ca++ entering renal cells during interaction of the peptide with its receptor is the source of cellular Ca++ involved in the prostaglandin synthesis-stimulating action of All.

An important finding in the present study was that trifluoperazine, W-7, and calmidazolium, in concentrations known to inhibit calmodulin activity (Levin and Weiss, 1976; Hidaka et al., 1981; Van Belle, 1981), attenuated the renal output of prostaglandins elicited by All, but not that caused by exogenous arachidonic acid. This finding, and the demonstration that a phospholipase A2-like activity...
was increased by Ca++-calmodulin and inhibited by trifluoperazine in platelets (Wong and Cheung, 1979) and in renal medullary slices (Craven and DeRubertis, 1983), suggests that Ca++ released from intracellular storage sites by All may stimulate deacylation by interacting with calmodulin. A Ca++-calmodulin-mediated lipase, which stimulates prostaglandin synthesis, has also been reported for arginine vasopressin in renal medullary slices (Craven and DeRubertis, 1981) and the isolated rat kidney (Cooper and Malik, 1984). It appears unlikely, from our study, that the reduction of All-induced prostaglandin output by calmodulin inhibitors is due to their interference with intracellular Ca++ release, since the effect of the calmodulin inhibitors to attenuate All-induced prostaglandin output was not altered by the addition of Ca++ to the medium, whereas the inhibitory effect of intracellular Ca++ antagonists on prostaglandin output elicited by All was abolished by raising the Ca++ concentration to 1.8 mM. The calmodulin inhibitors failed to alter the effect of BK to enhance renal output of prostaglandins, which suggests that BK stimulates prostaglandin synthesis in the rat kidney by a mechanism independent of calmodulin. However, Crutchley et al. (1983), in bovine pulmonary artery, and Whorton et al. (1982), in porcine aortic cultured endothelial cells, have reported that Ca++ depletion or Ca++ channel blockers and trifluoperazine attenuated BK-induced output of prostaglandins. It remains to be determined whether these differences in the susceptibility of BK-induced prostaglandin synthesis to Ca++ antagonists and calmodulin inhibitors in the rat kidney and endothelial cells, are due to differences in the cell type and/or the lipase activated by the kinin.

All and BK may release arachidonic acid for prostaglandin synthesis from tissue phospholipids by causing activation of an acylhydrolase, presumably phospholipase A2 or phospholipase C (Kunze and Vogt, 1971; Bell et al., 1979; Billah et al., 1981; Prescott and Majerus, 1983). Our demonstration that mepacrine, a phospholipase A2 and C inhibitor (Vargaftig and Coiron, 1971; Hoffmann et al., 1982), reduced the output of prostaglandins elicited by All and BK, but not that caused by exogenous arachidonic acid, suggests that All and BK stimulate prostaglandin synthesis by activating phospholipase A2 and/or C. However, the finding that intracellular Ca++ antagonists and calmodulin inhibitors attenuated All- but not BK-induced prostaglandin synthesis in the rat kidney suggests that the lipase involved in the action of All but not BK is dependent upon Ca++ and calmodulin. Further evidence suggesting that BK stimulates prostaglandin synthesis by a mechanism distinct from that of All resulted from our observation that the output of prostaglandins elicited by combined administration of All and BK was approximately equal to the sum of the prostaglandin output produced by administration of these peptides alone. Although mepacrine, in our study, inhibited All and BK-induced prostaglandin synthesis, it failed to alter the basal output of prostaglandins. Since, indomethacin, a cyclooxygenase inhibitor, reduced the basal and abolished All and BK-induced prostaglandin output, it appears that arachidonic acid released in the absence of hormone stimulation arises from a distinct lipid pool, or the lipase that stimulates deacylation is insensitive to All and BK (Schwartzman et al., 1981).

The present study in the isolated intact rat kidney indicates that All produces renal vasoconstriction by a mechanism that requires extracellular Ca++ but not calmodulin, whereas the effect of All to stimulate prostaglandin synthesis requires intracellular Ca++, which appears to interact with calmodulin and, presumably, activates phospholipase A2 and/or C. In contrast, BK enhances prostaglandin synthesis by stimulating a lipase that does not appear to require Ca++ and calmodulin.

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