Decreased Cytosolic Calcium and Prostaglandin Synthesis in Prehypertensive Rats

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The capacity of cultured renal medullary interstitial cells derived from Dahl salt-sensitive and salt-resistant rats to synthesize prostaglandin E₂ (PGE₂) was compared. Basal and arginine vasopressin (AVP)-induced PGE₂ production by interstitial cells from salt-resistant rats was fourfold to fivefold higher than corresponding values of those from the salt-sensitive rats. Similarly, basal and AVP-responsive release of [³H]arachidonate were twofold higher by interstitial cells from salt-resistant compared with salt-sensitive rats. Differences in PGE₂ production were abolished by the calcium inophore A23187 or the addition of exogenous arachidonate. The latter findings suggested a role for altered availability of endogenous arachidonate, possibly mediated by reduced calcium-responsive lipase activity. Basal and AVP-induced increases in cytosolic free calcium concentration, assessed by the aequorin method, were significantly lower in interstitial cells from salt-sensitive versus salt-resistant rats, further supporting a possible role for altered cellular calcium homeostasis. Studies of the potential contribution of various phospholipases and of triglyceride lipase to the release of arachidonate for PGE₂ synthesis in interstitial cells implicated phospholipase A₂ activity as a major pathway. When assessed in vitro in cell cytosolic fractions at identical calcium concentration, phospholipase A₂ activity was lower in interstitial cells from salt-sensitive versus salt-resistant rats. Thus, both reduced cytosolic free calcium and phospholipase A₂ activity of interstitial cells from salt-sensitive rats may contribute to the diminished capacity of these cells to liberate endogenous arachidonate for PGE₂ synthesis. (Hypertension 1990;15:388–396)

Previous studies in animal models of renal medullary ablation have demonstrated a potent antihypertensive action of the renal medulla against a number of hypertensive stimuli including a high salt diet. Transplantation of renomedullary interstitial cells grown in culture reduces blood pressure in animals with various forms of hypertension and further supports a role for the interstitial cell as a source of vasodepressor humoral agents. Studies in experimental genetic models of salt-induced hypertension such as the Dahl salt-sensitive (DS) and salt-resistant (DR) rat model suggest that inherited differences in the antihypertensive capacity of the renal medulla may explain the differences in the susceptibility of these rats to the development of high blood pressure when they are placed on a high salt diet.

Among the factors that have been suggested as contributing to the antihypertensive action of the renal medulla are prostaglandin E₂ (PGE₂) and antihypertensive polar renomedullary lipid (APRL), a member of a class of lipids, the 1-alkyl-2-acyl-glycerol-ether phosphorylcholines. The release of arachidonic acid for PGE₂ synthesis as well as release of APRL follows activation of cellular lipases and may be triggered by a rise in cytosolic free calcium ([Ca²⁺]).

Previous studies in DS and DR rats have demonstrated lower urinary PGE₂ excretion and PGE₂ content of quick-frozen renal medulla obtained from prehypertensive DS compared with DR rats. The differences in PGE₂ persist after the rats are placed on a high salt diet at which point the DS rats become hypertensive, whereas the DR rats remain normotensive. The mechanisms and cell types responsible for this decrease in renal medullary PGE₂ production have not been examined in detail. In the present study, we examined 1) the capacity of interstitial cells cultured from the renal medulla of DS or DR rats to synthesize PGE₂ basally and in response to vasopressin (AVP), the calcium ionophore A23187, and exogenous arachidonic acid; 2) the relative contribution of...
phospholipases A₂, C, and D and triglyceride lipase to the generation of arachidonic acid for PGE₂ production; and 3) the potential role of alterations in [Ca²⁺], in mediating the differences in arachidonate release and PGE₂ synthesis of medullary interstitial cells derived from DS and DR rats.

**Methods**

**Treatment of Rats**

Male DS and DR rats were obtained from Brookhaven National Laboratories (Upton, New York) at weaning (3 weeks of age) and were maintained on a low salt (0.07–0.09% Na⁺) diet until 6 weeks of age. Indirect tail-cuff measurements of blood pressure were obtained in conscious rats before they were killed. Four readings were obtained per rat.

**Culture of Interstitial Cells**

Interstitial cells were prepared from 6-week-old DS and DR rats for culture as previously described.¹,² The inner medulla from three rats was minced under sterile conditions with two scalpel blades. Approximately three to four pieces of minced medulla were obtained per kidney. The mince was pooled, mixed with tissue culture medium (basal Eagle’s medium plus glutamine [0.29 mg/ml], penicillin [100 units/ml], streptomycin [100 µg/ml], lactalbumin hydrolysate [2.5%, vol/vol], yeast extract [0.05%, vol/vol], amino acids, and 10% fetal bovine serum [FBS]) divided into two portions and injected subcutaneously with a trocar on the abdomen of two recipient rats. Donor and recipient rats were of the same type (either DS or DR rats). The wound was then closed with wound clips. Within 3–6 days, the nodules that formed at the injection site were removed aseptically, minced, and incubated twice successively with 5 ml Dulbecco’s phosphate buffered saline with penicillin (100 units/ml), streptomycin (100 µg/ml), and 0.8% trypsin for 30 minutes at 37°C. The suspension was centrifuged and washed in culture medium plus 10% FBS. The cells were plated in three 25 cm² Primaria tissue culture flasks. The cells were rinsed and incubated in fresh K-1 medium (0.5 ml/well). Interstitial cells were rinsed and incubated in 0.5 ml/well of Eagle’s basal medium plus glutamine, penicillin, and streptomycin. All incubations of RPCT or interstitial cells for PGE₂ determination were conducted with confluent cultures in 24-well plates at 37°C for the times and with test agents as indicated in the text. The gas phase was 7% CO₂, balance air. Inner medullary slice incubations were conducted in 2 ml Krebs-Ringer bicarbonate buffer (KRBB) in 25 ml flasks at 37°C for the times and with test agents as indicated in the text. The gas phase was 5% O₂, 5% CO₂, balance N₂. PGE₂ content of the final incubation media was determined by radioimmunoassay (DuPont, New England Nuclear, Boston, Massachusetts).

**Culture of Renal Papillary Collecting Tubule Epithelial Cells**

Renal papillary collecting tubule (RPCT) cells were prepared as previously described.¹,³ The inner medullary tissue from two to three rats was used to prepare enough cells for a single 24-well plate. The medullas were removed aseptically and minced finely to a paste with two scalpel blades. The paste was mixed with 4 ml Type II collagenase (1 mg/ml) (Cooper Biomedical, Inc., Malvern, Pennsylvania) plus 100 units/ml penicillin and 100 µg/ml streptomycin in phosphate buffered saline for 2 hours at 37°C, with gentle shaking every 15 minutes. At the end of 20 minutes, 6 ml sterile H₂O was added. Large pieces of tissue were allowed to settle and the supernatant centrifuged at 2,000g. The resulting cell pellet was washed with phosphate buffered saline containing 10% bovine serum albumin, resuspended in 12 ml tissue culture media and seeded into a 24-well plate. Tissue culture medium for plating was Dulbecco’s modified Eagle medium (DMEM)/F-12, penicillin (100 units/ml), streptomycin (100 µg/ml), and 10% FBS. After 24 hours, the medium was changed to defined K-1 epithelial growth medium.¹,³ Medium was changed daily. The cells were usually confluent after 3–4 days.

**Incubation of Cells and Assay of Prostaglandin E₂**

RPCT cells were rinsed and incubated in fresh K-1 medium (0.5 ml/well). Interstitial cells were rinsed and incubated in 0.5 ml/well of Eagle’s basal medium plus glutamine, penicillin, and streptomycin. All incubations of RPCT or interstitial cells for PGE₂ determination were conducted with confluent cultures in 24-well plates at 37°C for the times and with test agents as indicated in the text. The gas phase was 7% CO₂, balance air. Inner medullary slice incubations were conducted in 2 ml Krebs-Ringer bicarbonate buffer (KRBB) in 25 ml flasks at 37°C for the times and with test agents as indicated in the text. The gas phase was 5% O₂, 5% CO₂, balance N₂. PGE₂ content of the final incubation media was determined by radioimmunoassay (DuPont, New England Nuclear, Boston, Massachusetts).

**Interstitial Cell [Ca²⁺]**

Interstitial cells were grown to confluence in a 75 cm² tissue culture flask. The cells were rinsed in Ca²⁺, Mg²⁺-free phosphate buffered saline. Cold 0.8% trypsin (4 ml) in phosphate buffered saline was added and the trypsin solution removed after 10 seconds. The cells were incubated 10–15 minutes at 37°C and washed off the flask with 15 ml culture media containing 10% FBS. The cells were washed twice in 15 ml media plus FBS and then in 15 ml media without serum by centrifugation at 2,000 rpm for 1 minute before loading with aequorin. The procedure for loading aequorin¹⁴ and for measuring [Ca²⁺]¹⁵ were as previously described. Aequorin was purchased from Dr. J.R. Blinks (Mayo Foundation, Rochester, Minnesota). Interstitial cells, which had been incorporated into agarose threads,¹⁴,¹⁵ were suspended in an aequorin photometer and perfused with KRBB (95% O₂, 5% CO₂) at 1 ml/min for the times and with test agents as indicated in the text. The sample compartment was maintained at 37°C. By using this protocol, multiple determinations of [Ca²⁺], could be made with the same cell preparation under different experimental conditions. In the studies of the concentration-response relation between AVP and [Ca²⁺], the lowest concentration of AVP to be studied was tested first. Cells were perfused with AVP for
5 minutes followed by 30 minutes of perfusion with KRBG (95% O2, 5% CO2) before raising the concentration of AVP to the next highest dose. In the studies of the influence of the pressor antagonist of AVP, cells were perfused with the pressor antagonist first and the response to AVP determined. Cells were then perfused for 30 minutes with KRBG (95% O2, 5% CO2) and the response to AVP determined in the absence of the pressor antagonist. [Ca2+]i was derived from a curve of the negative log of [Ca2+]i in standard solutions versus the negative log of the observed fractional luminescence. Fractional luminescence was determined by dividing the observed luminescence by the maximal luminescence obtained when cells were perfused with 1% Triton X-100.15

Incubation and Extraction of Interstitial Cells for Determination of Effects of Arginine Vasopressin on Distribution of Labeled Arachidionate and Choline

Interstitial cells from DS and DR rats were grown to confluence in two 75 cm2 flasks each. They were labeled for 2 days with 5 μCi [3H]arachidonate and 5 μCi [3H]choline in 10 ml media for each flask. At the end of 1 day, uptake of the label into the cells had reached equilibrium as judged by the fact that the counts in the media were the same at 1 and 2 days. The percent of arachidonate taken up was 66% in DS rat cells and 69% in DR rat cells. Choline incorporation was also similar in the two strains (DS, 61%; DR, 64%). At the end of 2 days, the cells were washed and media changed to basal Eagle’s medium containing glutamine, penicillin, and streptomycin. AVP (10−6 M) was added to one of the flasks in each group and the incubation continued for 30 minutes. At the end of the incubation, the medium was removed and stored at −20°C for thin-layer chromatography. CH3OH (4 ml) was added to the cells, and cells were transferred to glass tubes to which were added 2 ml 1 M NaCl and 2 ml CHCl3. The extracts were stored at least 2 hours at −20°C; 2 ml CHCl3 and 2 ml 1 M NaCl were added to break the phases, and the CHCl3 was layer removed. The aqueous layer was extracted three more times with 1 ml CHCl3 each time. The CHCl3 layers were combined, evaporated to dryness, and the sample resuspended in 0.5 ml CHCl3 for thin-layer chromatography. Tritium counts in the media were assumed to be due to [3H]arachidonate and its metabolites. In some studies, where indicated in the text, an aliquot of the CHCl3 extract was used for the determination of phospholipid phosphorus as previously described.16

Thin-Layer Chromatography

Diacylglycerol, triacylglycerol, and arachidonate were separated by thin-layer chromatography of CHCl3 extracts on silica gel G thin-layer plates by using CHCl3/CH3OH/acetic acid/H2O (25/15/4/1.5).18 Sample spots were overlaid with 50 μCi each phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol. Visualization was with molybdenum blue spray reagent (1.3% molybdenum oxide in 4.2 M H2SO4).

Phosphatidic acid was separated from CHCl3 extracts on silica gel G with CHCl3/pyridine/70% formic acid (50/30/7).19 Sample spots were overlaid with 200 μg phosphatidic acid and visualized with molybdenum blue spray reagent.

For determination of choline, glycerol-3-phosphorylcholine, and phosphorylcholine, media and aqueous extracts of cells were applied to silica gel G thin-layer plates and overlaid with choline (50 μg), phosphorylcholine (300 μg), and glycerol-3-phosphorylcholine (300 μg). The running phase was saline/CH3OH/NH4OH (50/70/5).18 Visualization was with Dragendorf’s reagent. Spots were then scraped and counted and results expressed as a percentage of counts recovered in the CHCl3 plus aqueous layers.

Phospholipase A2

Interstitial cells from DS and DR rats were homogenized in 50 mM Tris, pH 9 (107 cells/3 ml) and centrifuged at 2,500 rpm in the Sorval SS-34 rotor (75Sg) for 15 minutes. The 75Sg supernatant was used for assay. Assay mixtures contained 50 mM Tris, pH 9, 5 mM CaCl2, 1 mg/ml sodium deoxycholate, 100 μM phosphatidylcholine, and 200,000 disintegrations per minute of each phosphatidylethanolamine, 1,1-2-dipalmitoyl [choline-methyl-[3H]] and phosphatidylethanolamine-1-palmitoyl-2-arachidonoyl, [arachidonoyl-1-13C] in a final volume of 2 ml. Reactions were conducted for 60 minutes at 37°C and stopped by the addition of 50 μl formic acid plus 8 μl CHCl3/CH3OH (2/1). Lysophosphatidylethanolamine and phosphatidylethanolamine were separated by thin-layer chromatography on silica gel G by using CHCl3/CH3OH/acetic acid/H2O (25/15/4/1.5) as described above. Arachidonic acid was separated from phosphatidylethanolamine by thin-layer chromatography on silica gel G by using hexane/ether/acetic acid (80/20/4) as described above. Spots were counted by using a dual label program and results expressed as the picomoles of [3H]arachidonate or [3H]lysophosphatidylethanolamine formed from phosphatidylethanolamine per minute per 106 cells.

Statistics

The significance of differences between any two mean values was determined by Student’s independent t test. Experiments were conducted three or four times as indicated in the footnotes to the tables and figures.
Table 1. Prostaglandin E2 Production by Inner Medullary Slices and Renal Papillary Collecting Tubule Epithelial Cells from Dahl Salt-Sensitive and Salt-Resistant Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Inner medullary slices (ng/mg protein/30 min)</th>
<th>RPCT cells (ng/mg protein/30 min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Basal (1 μM)</td>
<td>AVP (10 μM)</td>
</tr>
<tr>
<td>DS</td>
<td>2.1 ±0.3</td>
<td>6.4 ±0.9</td>
</tr>
<tr>
<td>DR</td>
<td>5.2* ±0.8</td>
<td>25* ±3</td>
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Inner medullary slices were incubated for 150 minutes in Krebs-Ringer bicarbonate buffer (5% O2, 5% CO2) with media changes at t=60 and 120 minutes. Where indicated arginine vasopressin (AVP) or arachidonate were added at t=120 minutes and media removed for prostaglandin E2 (PGE2) determination at t=150 minutes. Renal papillary collecting tubule epithelial (RPCT) cells were grown to confluence in primary culture as described in Methods. They were washed and incubated in KRBG, 7% O2 (balance air). Where indicated, AVP or arachidonate was added for 30 minutes and media removed for assay of PGE2. Results shown are mean±SEM of determinations from four separate experiments. DS, Dahl salt-sensitive rats; DR, Dahl salt-resistant rats.

*p<0.05 vs. values in DS.

Results

Table 1 illustrates PGE2 production by inner medullary slices and RPCT cells obtained from DS and DR rats. All rats were maintained on a low salt diet (0.07–0.09% Na+) and then killed at 6 weeks of age. Blood pressure in both groups of rats, as determined by the tail-cuff method, was normal (DS, 105±4; DR, 108±3 mm Hg). Basal PGE2 production was 1.5-fold to twofold higher in inner medullary slices and RPCT cells prepared from DR versus DS rats. AVP-induced increases in PGE2 were also greater in medullary slices from DR versus DS rats. Consistent with previous results, AVP did not increase PGE2 in RPCT cells. Arachidonate increased PGE2 production to the same absolute level in medullary slices from DR versus DS rats thus suggesting that altered availability of endogenous arachidonate may be the basis for the differences in medullary PGE2 synthesis. By contrast, and consistent with previous studies, arachidonate-induced increases in PGE2 were significantly lower in RPCT cells from DS versus DR rats.

As shown in Figure 1, cultured medullary interstitial cells from DS and DR rats produced 30 and 100 times, respectively, more PGE2 basally compared with cultured RPCT cells when expressed on the basis of protein. Moreover, basal and AVP-induced increases in PGE2 were fourfold to fivefold higher in interstitial cells from DR versus DS rats. However, as is also shown in Figure 1, addition of A23187 or arachidonate increased PGE2 to the same absolute value in interstitial cells from DS compared with DR rats.

Figure 2 shows the concentration-response relation between AVP and release of [3H]arachidionate from interstitial cells of DS and DR rats. AVP (10^-7 to 10^-4 M) increased [3H]arachidionate release by interstitial cells from both DS and DR rats. However, basal [3H]arachidionate release and the stimulation by AVP was twofold higher by interstitial cells from DR versus DS rats.

Figure 3 illustrates the effects of AVP on [Ca2+] in interstitial cells from DR rats. Cells were dissociated with trypsin, loaded with aequorin, incorporated into agarose threads, and perfused with KRBG (95% O2, 5% CO2) at a rate of 1 ml/min. AVP (10^-7 M) was added to the perfusate at the time indicated by the arrow. A representative tracing is shown in Figure 3. The increase in luminescence shown represents a change from a baseline of 205 nM to 730 nM [Ca2+].

Figure 4 shows the concentration-response relation between AVP and [Ca2+] in DS versus DR rats. Basal [Ca2+] was twofold higher in interstitial cells from DR versus DS rats. AVP increased [Ca2+] in cells from both DS and DR rats. Optimal effects were observed at 10^-7 M in interstitial cells from both groups of rats. However, the threshold dose of AVP, which increased calcium, was much lower (10^-9 M) in interstitial cells from DR versus DS (10^-7 M) rats. Moreover, the absolute level of [Ca2+] achieved was markedly higher in interstitial cells from DR versus
FIGURE 2. Line graph showing concentration-response relation between arginine vasopressin (AVP) and release of [3H]arachidonate from interstitial cells of Dahl salt-sensitive (Dahl-S) and Dahl salt-resistant (Dahl-R) rats. Interstitial cells grown to confluence in 24-well plates were prelabeled for 24 hours with [3H]arachidonate, washed to remove excess label and incubated with the indicated concentrations of AVP. [3H]Arachidonate, released into the media, was assessed 30 minutes after addition of AVP. Results shown are mean±SEM of determinations from three separate experiments. All values obtained with interstitial cells from Dahl-R rats were significantly different from those obtained with cells from Dahl-S rats. *p<0.05 vs. basal.

FIGURE 3. Representative tracing showing effects of arginine vasopressin (AVP) on cytosolic calcium ([Ca2+]i) of interstitial cells from Dahl salt-resistant (Dahl-R) rats. [Ca2+]i was measured in interstitial cells that had been loaded with aequorin. Cells were incorporated into agarose threads and perfused with Kreb's KRBG, 95% O2, 5% CO2 at a rate of 1 ml/min. AVP was added where indicated by arrow.

DS rats at all concentrations of AVP tested (10^{-9} to 10^{-8} M). In other studies (not shown) indomethacin (50 μM), which inhibited basal and AVP-responsive PGE2 by 75–80%, had no effect on basal or AVP-induced increases in [Ca2+]i in interstitial cells from DR or DS rats. Moreover, addition of PGE2 (10^{-6} M) to the perfusate had no effect on the peak calcium response obtained at each AVP concentration with four different cell preparations. *p<0.05 vs. basal. #p<0.05 vs. values obtained with cells from Dahl-S rats.

In other studies (not shown), we examined the effects of the pressor antagonist, [1-d-(CH2)5, Tyr(Me)]-AVP (10^{-5} M) on basal and AVP (10^{-7} M)-induced increases in PGE2 and [Ca2+]i in interstitial cells from DR and DS rats. The pressor antagonist had no effect on basal [Ca2+]i, or PGE2 but completely blocked increases in these parameters induced by AVP. The ability of the pressor antagonist to block AVP-induced increases in interstitial cell PGE2 production is consistent with previous studies.13

The release of arachidonate for PGE2 synthesis may occur through phospholipase A2, C, or D or triacylglycerol lipase action. To gain insight into the pathways that may mediate AVP-induced release of arachidonate for PGE2 synthesis, interstitial cells from DS and DR rats were prelabeled with [3H]arachidonate and [14C]choline chloride for 3 days, washed, and incubated for 30 minutes with or without 10^{-7} M AVP. In these studies, phospholipids were assumed to be labeled to a constant specific activity as determined by the fact that the uptake of [14C]choline and [3H]arachidonate into phospholipids did not change between days 2 and 3 of incubation with the labeled compounds when expressed as a function of lipid phosphorus (not shown). The distribution of [3H]arachidonate and [14C]choline among lipid and water soluble metabolites is shown in
FIGURE 5. Bar graph showing effects of arginine vasopressin (AVP) on distribution of [3H]arachidonate among interstitial cell lipids from Dahl salt-sensitive (Dahl-S) and Dahl salt-resistant (Dahl-R) rats. Interstitial cells from Dahl-S and Dahl-R rats were prelabeled with [3H]arachidonate for 3 days and incubated for 30 minutes with (•) or without (○) 10^{-7} M AVP. Distribution among lipid and water soluble metabolites of [3H]arachidonate is shown. AA, arachidonate; TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylserine; PS, phosphatidylserine; PI, phosphatidylinositol. Results shown are mean±SEM of duplicate extractions from two separate experiments. Counts per minute recovered in each moiety are expressed as a percent of the total counts per minute recovered in cells plus media. Total counts per minute recovered was not significantly different in extracts from Dahl-S vs. Dahl-R rats. *p<0.05 vs. results obtained in absence of AVP.

FIGURE 6. Bar graph showing effects of arginine vasopressin (AVP) on distribution of [14C]choline among interstitial cell lipids from Dahl salt-sensitive (Dahl-S) and Dahl salt-resistant (Dahl-R) rats. Interstitial cells from Dahl-S and Dahl-R rats were prelabeled with [14C]choline for 3 days and incubated for 30 minutes with (•) or without (○) 10^{-7} M AVP. Distribution among lipid and water soluble metabolites of [14C]choline is shown. PC, phosphatidylcholine; G-3PC, glycerol 3-phosphorylcholine; LPC, lysophosphatidylcholine; Chol-P, phosphorylcholine; Choi, choline.

The distribution of [14C]choline among phosphatidylcholine, glycerol-3-phosphorylcholine, lysophosphatidylcholine, phosphorylcholine, and choline is shown in Figure 6. AVP markedly increased the formation of labeled glycerol-3-phosphorylcholine in interstitial cells from DR rats but had a much smaller effect on glycerol-3-phosphorylcholine accumulation in cells from DS rats. AVP also modestly increased labeled phosphorylcholine formation in interstitial cells from DR but not DS rats. Of note, AVP did not detectably increase choline-labeled lysophosphatidylcholine formation. These findings imply the existence of a lysophospholipase activity in the interstitial cells. The presence of lysophospholipase activity was also supported by studies of phospholipase activity in interstitial cell homogenates that were incubated with labeled exogenous phosphatidylcholine (see below). Glycerol-3-phosphorylcholine is formed by the combined action of phospholipases A_{2} or A_{1} plus lysophospholipase activity. The large increase in glycerol-3-phosphorylcholine compared with the much smaller increase in phosphorylcholine implies that the predominant mode of AVP-induced metabolism of phosphatidylcholine in interstitial cells is via phospholipase A plus lysophospholipase with a lesser contribution from the phospholipase C pathway. The results also suggest that the higher levels of AVP-
stimulated arachidonate release and PGE2 synthesis seen in interstitial cells from DR versus DS rats involve increased phospholipase A2 activity. The failure of AVP to increase [14C]choline release suggests that the phospholipase D pathway does not contribute significantly to AVP-stimulated phosphatidylcholine hydrolysis in interstitial cells.

In the present study, the release of choline-labeled glycerol-3-phosphorylcholine in response to AVP was more than sufficient to account for the release of labeled arachidonate observed, supporting an action of phospholipase A2 on phosphatidylcholine. Nevertheless, we did not specifically assess the contribution of other phospholipids, including phosphatidylinositol, to arachidonate release and cannot rule out a contribution from these lipids.

Table 2 compares phospholipase A2 activity in homogenates of interstitial cells from DS and DR rats. Reaction mixtures contained phosphatidylcholine labeled with tritium in the choline moiety as well as that labeled with carbon-14 in the arachidonate portion of the molecule. Both labels were used in the present study to distinguish between phospholipases A2, A3, C, and D. In preliminary experiments conducted in the absence of deoxycholate, all of the labeled choline derived from phosphatidylcholine was recovered in glycerol-3-phosphorylcholine. By contrast, in the presence of deoxycholate, all of the labeled choline lost from phosphatidylcholine was recovered in lysophosphatidylcholine. These results are consistent with previous studies in fresh inner medullary tissue.21 These studies thus clearly establish that the ability of interstitial cells to release arachidonate: 1) PGE2 production was reduced basally and in the presence of AVP, whereas addition of exogenous arachidonate increased PGE2 production to the same absolute level in interstitial cells from both groups of rats; 2) release of labeled arachidonate was higher basally and in response to AVP in prelabeled interstitial cells from DR versus DS rats; and 3) phospholipase A2 activity was higher in interstitial cell homogenates from DR versus DS rats. The finding of higher phospholipase A2 activity in interstitial cells from DR versus DS rats is consistent with previous studies in fresh medullary tissue.21 These studies thus clearly support a role for differences in the capacity of the cells to release arachidonate rather than in the ability of the cells to convert arachidonate to PGE2 in the mediation of lower PGE2 production by interstitial cells from DS versus DR rats. The findings in interstitial cells are in contrast to results previously reported in RPCT cells; PGE2 production was reduced in RPCT cells from DS versus DR rats in the presence as well as in the absence of exogenous arachidonate.13 (Table 1). The latter implied lower prostaglandin synthetic capacity in the mediation of

Phospholipase A2 activity was determined in homogenates of interstitial cells from Dahl salt-sensitive (DS) and salt-resistant (DR) rats as described in Methods. Results shown are mean±SEM of determinations from three separate experiments. *p<0.05 compared with values for DS rats.

<table>
<thead>
<tr>
<th>Rat group</th>
<th>[14C]Arachidonate (pmol/min/10^6 cells)</th>
<th>[3H]Lysophosphatidylcholine (pmol/min/10^6 cells)</th>
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<tr>
<td>DS</td>
<td>9.8±2</td>
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<thead>
<tr>
<th>Rat group</th>
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<tr>
<td>DS</td>
<td>9.8±2</td>
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<tr>
<td>DR</td>
<td>23±4</td>
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**Discussion**

The results of the present study demonstrate that the ability of cultured interstitial cells from DS rats to release arachidonate via the phospholipase A2 pathway for the synthesis of PGE2 is reduced compared with that of cells from the DR rat. The difference in arachidonate release persisted through at least 20 passages and was observed in freshly prepared slices of renal medullary tissue, suggesting that it was not an artifact of the cell culture procedure. The results support the presence of a constitutional difference in the ability of interstitial cells from DS compared with DR rats to generate arachidonic acid through the phospholipase A2 pathway. Because the medullary tissue used in these studies was obtained from normotensive DS and DR rats fed a low salt diet, secondary effects induced by hypertension per se were excluded.

Prostaglandins produced in the kidney are thought to play a key role in the control of blood pressure in response to a wide variety of hypertensive stimuli. Enhanced renal medullary PGE2 production may protect the DR rat from the development of high blood pressure when placed on a high salt diet by facilitating salt and water excretion and enhancing medullary blood flow. In this regard, it has recently been demonstrated that feeding DS rats a diet rich in linoleic acid, which resulted in a significant increase in medullary PGE2 production, protected the rats from the development of high blood pressure when placed on a high salt diet. Several observations from our studies suggest that the defect in PGE2 synthetic capacity of interstitial cells from DS versus DR rats is due to a defect in the ability of these cells to release arachidonate: 1) PGE2 production was reduced basally and in the presence of AVP, whereas addition of exogenous arachidonate increased PGE2 production to the same absolute level in interstitial cells from both groups of rats; 2) release of labeled arachidonate was higher basally and in response to AVP in prelabeled interstitial cells from DR versus DS rats; and 3) phospholipase A2 activity was higher in interstitial cell homogenates from DR versus DS rats. The finding of higher phospholipase A2 activity in interstitial cells from DR versus DS rats is consistent with previous studies in fresh medullary tissue. These studies thus clearly support a role for differences in the capacity of the cells to release arachidonate rather than in the ability of the cells to convert arachidonate to PGE2 in the mediation of lower PGE2 production by interstitial cells from DS versus DR rats. The findings in interstitial cells are in contrast to results previously reported in RPCT cells; PGE2 production was reduced in RPCT cells from DS versus DR rats in the presence as well as in the absence of exogenous arachidonate (Table 1). The latter implied lower prostaglandin synthetic capacity in the mediation of
this effect. The results obtained in interstitial cells are analogous to those obtained in freshly prepared medullary slices, probably because of the fact that the interstitial cells are the major source of PGE₂ produced by whole medullary tissue.

The present studies demonstrated significantly higher basal and AVP-induced increases in [Ca²⁺] in interstitial cells from DR versus DS rats. Studies with the calcium inophore A23187 suggested that the differences in basal and AVP-responsive [Ca²⁺], might account for the higher levels of arachidonate release and PGE₂ observed in interstitial cells from DR versus DS rats. Thus, the addition of A23187 increased PGE₂ to the same absolute level in interstitial cells from DR and DS rats and abolished the differences between the two groups of rats. However, with respect to the blunted AVP responses observed in the DS rats, alterations in AVP receptor sites or in guanine nucleotide binding protein interactions were not excluded and could have contributed to the loss of AVP responsiveness. Moreover, as phospholipase A₂ activity in homogenates of interstitial cells from DR rats was higher than that in cells from DS rats when assessed at the same concentration of calcium, the possibility that higher levels of phospholipase A₂ activity also contributed to the increase in arachidonate release and PGE₂ seen in DR versus DS rats cannot be excluded. Previous studies have demonstrated that AVP increases [Ca²⁺] in cortical collecting tubule cells through a V₁ receptor but acts through a V₂ receptor in RPCT. The present study demonstrates that AVP increases both [Ca²⁺], and PGE₂ through a V₂ receptor in interstitial cells, consistent with a relation between the increase in [Ca²⁺], and the increase in PGE₂.

The mechanisms responsible for the higher [Ca²⁺], levels in interstitial cells from DR versus DS rats are unclear. However, the persistence of this difference through multiple cell passages again suggests a genetic basis. Our findings did not support an action of PGE₂ or other cyclooxygenase products to mediate the differences in [Ca²⁺],. Thus, PGE₂ per se did not increase [Ca²⁺], in cells from DS or DR rats, and indomethacin failed to suppress [Ca²⁺], in cells from the DR or DS rats.

Previous studies have described increases in basal or agonist-responsive [Ca²⁺], in platelets and vascular smooth muscle cells isolated from hypertensive patients or rats. The present study is the first to describe a reduction in [Ca²⁺], in cells from prehypertensive rats. It has been proposed that the release of a circulating inhibitor of sodium-potassium adenosine triphosphatase (Na⁺,K⁺-ATPase) in response to Na⁺ retention may mediate the increase in [Ca²⁺], of vascular smooth muscle cells through suppression of Na⁺-Ca²⁺ exchange. However, several recent reports have suggested that an intrinsic membrane defect may contribute to the alteration in cellular calcium handling observed in hypertension. Thus, studies in platelets and vascular smooth muscle from hypertensive rats or patients have described increases in phospholipase C, inositol phospholipid turnover, and protein kinase C activity. By contrast, decreases in inositol phospholipid turnover have been reported in erythrocytes and rat renal cortex from hypertensive rats. Other studies have correlated increased [Ca²⁺], in experimental and clinical forms of primary hypertension with altered passive calcium diffusion, Ca²⁺ pump activity, and calcium binding proteins. In some studies, altered membrane properties were evident in prehypertensive rats. Moreover, in one previous report basal [Ca²⁺], remained higher than normal in vascular smooth muscle cells obtained from spontaneously hypertensive rats and cultured through six passages. The latter study describes an increase in [Ca²⁺], in cultured vascular smooth muscle cells from spontaneously hypertensive rats, whereas we have observed a decrease in [Ca²⁺], in cultured interstitial cells from prehypertensive rats. Nevertheless, a finding common to both studies is that the maintenance of an alteration in basal [Ca²⁺], was not the consequence of an increase in blood pressure and was not mediated by a humoral factor, but may at least in part reflect a genetically determined cellular property.

Previous reports have described the existence of a humoral renomedullary system for blood pressure control that involves the release of APRL. The latter belongs to a class of molecules known as 1-alkyl-2-acyl-glyceryl-ether phosphorylcholines. The release of APRL from membrane lipids is thought to be initiated by calcium-dependent phospholipase A₂ activity and may occur concurrently with arachidonate release. APRL is a potent hypotensive agent when infused in rats and has been implicated as the mediator of the reduction in blood pressure that occurs after unclipping the artery in the two-kidney, one clip model of Goldblatt hypertension. The role of APRL in the hypotensive function of the renal medulla in salt-induced hypertension and its possible interrelation with PGE₂ is not known. However, the present results, which demonstrate higher [Ca²⁺], and phospholipase A₂ activity in interstitial cells from DR versus DS rats, raises the possibility that the APRL-generating capacity of interstitial cells from DS rats may also be impaired.

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