Aldosterone Enhances Angiotensin II Receptor Binding and Inositol Phosphate Responses

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Clinical states in which angiotensin II is increased are often associated with increases in mineralocorticoids. To determine the effects of aldosterone on angiotensin II action, we examined the effects of aldosterone on angiotensin II receptor expression and function in cultured rat vascular smooth muscle cells. Incubation with aldosterone resulted in concentration- and time-dependent increases in angiotensin II receptor number, without changes in binding affinity. For example, incubation with 1 μM aldosterone for 40 hours resulted in 59% increases in angiotensin II receptor number. Increases in angiotensin II receptors were dependent on protein synthesis as evidenced by the time dependency of upregulation and inhibition by cycloheximide. Incubation with aldosterone resulted in enhanced angiotensin II-stimulated phospholipase C activation, as demonstrated by increases in angiotensin II-induced inositol phosphate responses in proportion to the increases in receptor number. In addition, aldosterone prevented angiotensin II-induced downregulation of angiotensin II surface receptors and angiotensin II desensitization of inositol phosphate formation. In summary, aldosterone 1) directly increased angiotensin II receptor number, 2) increased angiotensin II-stimulated inositol phosphate responses, and 3) prevented angiotensin II-induced downregulation and desensitization. In conclusion, aldosterone may potentiate the pressor responses of angiotensin II via effects on angiotensin II receptors. (Hypertension 1992;20:67–73)

KEY WORDS • aldosterone • angiotensin II • inositol phosphates • muscle, smooth, vascular

The pressor action of the potent vasoconstrictor angiotensin II (Ang II) is initiated by binding to specific receptors on the surface of vascular smooth muscle cells (VSMC). Ang II receptor complexes are coupled to phospholipase C, which results in the hydrolysis of phosphoinositides to diacylglycerol and inositol trisphosphate (IP3). Formation of IP3 mediates at least a portion of the Ang II pressor response by mobilizing calcium from intracellular stores.

A number of circulating factors have been shown to modulate Ang II surface receptor binding. These include Ang II, potassium, estrogen, catecholamines, glucocorticoids, testosterone, and mineralocorticoids. The role of mineralocorticoids in regulating Ang II binding is of particular importance in light of data suggesting that mineralocorticoids heighten the systemic pressor response to systemically administered Ang II.

Although in vivo studies indicate that mineralocorticoids increase Ang II binding to vascular tissue, the mechanisms of this effect are uncertain. In this regard, administration of mineralocorticoids results in potassium depletion and decreases in levels of circulating Ang II. Since both potassium depletion and decreases in Ang II are associated with increases in Ang II binding in vascular tissue, it is not possible to determine from in vivo studies whether increases in Ang II binding are mediated directly by mineralocorticoids or indirectly by potassium depletion or decreases in Ang II, or both. Use of cultured cells allows examination of the direct effects of mineralocorticoids on Ang II binding. In one study, incubation of cultured VSMC with a single concentration of the mineralocorticoid aldosterone (Aldo) resulted in increases in Ang II binding. In addition, concentration-dependent upregulation of Ang II receptor number in neuronal cultures by deoxycorticosterone acetate was found.

The functional consequences of increased Ang II binding to VSMC are not known. Since decreases in the number of Ang II receptors were associated with comparable decreases in Ang II–generated IP3 formation, tight coupling between Ang II receptors and Ang II–stimulated inositol phosphate (IP3) formation has been suggested. However, the relation between increases in Ang II receptors and Ang II–generated IP3 formation has not been determined under any circumstance. Studies of this relation will help to determine if tight coupling between Ang II receptors and Ang II–stimulated signal transduction persists over a wide range of receptor number.

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The purposes of our study, therefore, were 1) to determine the direct effects of Aldo independent of potassium or Ang II on Ang II receptor number in cultured VSMC and 2) to determine the effects of Aldo-induced increases in Ang II binding on Ang II–stimulated IP responses.

Methods

Vascular Smooth Muscle Cell Isolation and Maintenance

Experiments were performed on VSMC isolated from mesenteric arteries of male Sprague-Dawley rats (weight, 225–250 g) maintained on standard chow and tap water. After the rats were killed, the superior mesenteric artery with its major branches was excised. The techniques for isolation of mesenteric artery VSMC are modifications of those described by Ives et al and Gunther et al and have been described in detail by us previously. Superior mesenteric artery arcades were placed in ice-cold minimum essential medium with Earle’s salts. Fat, adventitia, and venous structures were removed by blunt dissection, and mesenteric arteries (usually three per preparation) were transferred into a flask containing 5.0 ml minimal essential medium with 0.25 mg/ml elastase, 0.75 mg/ml soybean trypsin inhibitor, 1.0 mg/ml collagenase, and 2.0 mg/ml crystallized bovine albumin. After incubation at 37°C for 120 minutes in a shaking water bath, the tissue suspension was aspirated with a Pasteur pipette and passed through a 100-μm nylon mesh to separate dispersed cells from undigested vessel wall fragments and debris. The filtered suspension was centrifuged in conical tubes (200g, 10 minutes), and cell pellets were resuspended in 10 ml growth medium: 10% (vol/vol) bovine calf serum and 1% (vol/vol) nonessential amino acids in minimal essential medium, plus 100 units/ml penicillin, 100 μg/ml streptomycin. The dispersed cell suspension was aliquoted into 25-cm² tissue culture flasks, which were incubated at 37°C for 120 minutes in a shaking water bath, the filtered suspension was centrifuged at 37°C in a humified 5% CO₂–95% air atmosphere. Growth medium was changed every 5 days. Cells were passaged every 7–10 days by harvesting with trypsin-EDTA and seeded at a ratio of 1:4.

Angiotensin II Binding

VSMC were passaged with a single load of a sterile repeater pipette to 24-well culture plates. This method of cell plating reduced the variability of well protein content to less than 5%. Equilibrium binding studies were done on cells at confluence, 4 to 7 days after plating, by methods originally described by Gunther et al and Penit et al. Assay buffer consisted of 50 mM Tris (pH 7.4), 100 mM NaCl, 5 mM MgCl₂, 0.25% bovine serum albumin, and 0.5 mg/ml bacitracin. At the beginning of each experiment, culture medium was aspirated from the wells and cells were washed twice with ice-cold saline. In most binding studies, 50 fmol of Ang II (50 fmol) was added to all wells, with or without varying concentrations of unlabeled Ang II (0.1–10 nM; 1 μM for nonsaturable binding). The incubation volume of each well was 0.3 ml. Binding conditions were 4°C for 90 minutes. Incubation was terminated by rapid removal of the medium and addition of 1 ml ice-cold saline. Free hormone was removed by washing the intact attached cells five times with ice-cold saline. Cells were then covered with 1% sodium dodecyl sulfate, detached by scraping with a rubber policeman, and transferred to a test tube, and gamma radioactivity was counted. Incubation with ethanol (0.1% or less), the vehicle for Aldo and spironolactone, for 40 hours did not alter Ang II binding.

Ang II binding to VSMC was time-dependent and confined to the cell surface. We have previously reported that Scatchard transformation of the binding data revealed a single class of receptors, with receptor density of 67±7 fmol/mg cell protein and a binding affinity of 2.14±0.14 nM at 4°C. Binding parameters were stable, and studies were performed in cells from passages 3 through 10. In the present study, single concentration binding ranged from 4 to 8 fmol/well in control wells.

Acid Washing

The acid washing technique of Crozat et al was used to remove Ang II from cell surface receptors. In preliminary experiments, 125I-Ang II was bound to cell surface sites at 4°C. Then cells were exposed to 150 mM NaCl, 50 mM glucose, pH 3, for 10 minutes at 4°C. After three saline washes, cell-associated radioactivity was reduced to the level of nonsaturable (nonspecific) binding. Subsequent 125I-Ang II binding could be restored to 100% of values obtained before acid washing.

Phospholipid Labeling and Measurement of Inositol Phosphates

These methods were adapted from Downes and Michell. VSMC were passaged with a sterile repeater pipette to six-well plates. After 4 days, the medium was changed to inositol-deficient growth medium with 2 μCi/ml myo-[4-3H]inositol (2 ml per well). Preliminary studies revealed that steady-state incorporation of radioactive inositol occurred after 24 hours. Then unincorporated tracer was removed by three saline washes at 21°C. After exposure to hormone or buffer (1 ml per well, 21°C, for 30 seconds), the reaction was terminated by the addition of 1 ml ice-cold 20% trichloracetic acid. Cells were then scraped with a rubber policeman, and the entire content of the well was transferred to a glass test tube. After centrifugation (400g for 10 minutes), the protein precipitate was discarded, and the supernatant was extracted three times with equal volumes of diethyl ether. The upper ether phase was discarded, and samples were adjusted to pH 6–7 with 50 mM Tris base and transferred to 2-cm columns of AG1-X8 anion exchange resin at 21°C. Radioactivity elutable with water and with borax (5 mM sodium borate and 60 mM sodium formate) was discarded. [3H]Inositol monophosphate ([3H]IP₁) was eluted with 0.2 M ammonium formate in 0.1 formic acid and discarded. Ammonium formate (0.5 M) in formic acid (0.1 M) eluted inositol bisphosphate (IP₂), and 1.0 M ammonium formate in 0.1 formic acid eluted IP₃. Each fraction was suspended in scintillation
fluid and counted in a scintillation counter. Basal values for IP, and IP, were 975±16 and 510±23 cpm per well, respectively. IP responses were calculated as the IP content in cells exposed to hormone compared with the IP content in cells exposed to buffer alone. Incubation with ethanol (0.1% or less), the vehicle for Aldo and spironolactone, for 40 hours did not elicit IP formation or alter Ang II-stimulated IP formation.

Protein Content

Cells were washed twice with saline and covered with 0.3 ml of 1% sodium dodecyl sulfate. The assay was adapted from Lowry, with a minor modification: absorbancy was read at 660 rather than 750 nm. Incubation with ethanol (0.1% or less), the vehicle for Aldo and spironolactone, for 40 hours did not alter cell protein content.

Measurement of Intracellular Potassium

Cells were washed four times with 4 ml of 100 mM MgCl$_2$ at 4°C. The cells were air dried and solubilized, and potassium of cell lysates were determined as previously described.

Thymidine Incorporation

Cells were plated on 24-well culture plates. Hormone and 2 μCi [3H]thymidine were added to each well as described in “Methods.” After incubation, the medium was aspirated and the cell layer washed twice with saline. Perchloric acid (0.5 ml of 0.3 M) was placed on the cells for 1 minute. Then the cells were washed once with saline and solubilized in 1 ml of 0.1N NaOH/0.1% sodium dodecyl sulfate. Scintillation fluid (10 ml) was added and radioactivity measured. Incubation with ethanol (0.1% or less), the vehicle for Aldo and spironolactone, for 40 hours did not alter thymidine incorporation.

Statistics

Results were expressed as the mean±SEM. Comparisons were made by paired or unpaired Student’s t test or one-way analysis of variance as described in the table and figure legends. For binding, $n$ refers to the number of triplicate groups of control and experimental wells. For IP, protein content, and thymidine incorporation studies, $n$ refers to the number of single control and experimental wells. Linearity of data was assessed by the least-squares method.

Materials

Materials were obtained from the following sources: Sigma Chemical Co., St. Louis, Mo., trypsin-EDTA, minimal essential medium, nonessential amino acids, Ang II, penicillin-streptomycin, elastase, soybean trypsin inhibitor, aldosterone, spironolactone, cycloheximide; New England Nuclear, Boston, Mass., 125-I-Ang II and [3H]thymidine; Hazelton Research Products, Denver, Pa., inositol-deficient minimal essential medium; HyClone Laboratories, Logan, Utah, bovine calf serum; Sasco Co., Omaha, Neb., Sprague-Dawley rats; ICN Pharmaceuticals, Plainview, N.Y., bovine serum albumin; Upjohn Co., Kalamazoo, Mich., bacitracin; American Radiolabeled Chemicals, St. Louis, Mo., [3H]myo-inositol; Biorad Laboratories, Richmond, Calif., Dowex AG1-X8 anion exchange resin; Packard Co., Downers

FIGURE 1. Scatterplot shows regulation of angiotensin II (Ang II) binding by aldosterone (Aldo). Confluent monolayers of cells were incubated with varying concentrations of Aldo in growth medium for 3 hours (○, n=3), 15 hours (□, n=3), or 40 hours (■, n=4–8) at 37°C. After Aldo was removed, Ang II binding at a single concentration of 125-I-Ang II was performed, as described in “Methods.” Data were calculated by comparing binding from cells incubated with Aldo to binding from control cells (not incubated with Aldo). Dashed line represents binding in control cells. *$p<0.05$ for linear regression analysis of 40-hour data.

Grove, III., Optifluor scintillation fluid; Cooper Biomedical, Malvern, Pa., collagenase.

Results

Effect of Incubation With Aldosterone on Angiotensin II Binding

VSMC were incubated with Aldo in growth medium at 37°C for 40 hours. Then Ang II binding at 4°C was performed. As shown in Figure 1, incubation with Aldo resulted in concentration-dependent increases in Ang II binding, with a maximum increase (59±7%) at 1 μM Aldo. Incubation with Aldo for 15 hours also resulted in increases in Ang II binding, which were less than after incubation for 40 hours. For example, incubation with 1 μM Aldo for 15 hours elicited 49±7% increase in Ang II binding. No increases in Ang II binding were observed after incubation with 1 μM Aldo for 3 hours. In preliminary studies, we found that including 1 μM Aldo in the binding assay only (i.e., not preincubated) did not alter Ang II binding. To determine if Aldo-induced increases in Ang II binding were mediated by changes in the number or affinity of Ang II receptors, full Ang II binding studies were performed after incubation with 100 nM Aldo for 15 hours. Incubation with Aldo resulted in increases in the number of Ang II receptors: 25±3 (control) versus 35±5 (Aldo) fmol/mg protein, $n=5$, $p<0.05$ by paired t test. In contrast, the apparent binding affinity was unchanged: 5.2±0.7 (control) versus 5.2±0.9 (Aldo) nM, $n=5$.

To determine if Aldo-induced increases in Ang II receptor number were mediated through the Aldo receptor, studies were repeated in the presence of the Aldo receptor antagonist spironolactone. Cells were incubated at 37°C for 40 hours with 10 nM Aldo alone or in the presence of 1 μM spironolactone. Concomitant administration of spironolactone reduced Aldo-induced
Angiotensin H-Induced Inositol Phosphate Formation: Effects of Incubation With Aldosterone on Angiotensin II-Stimulated Phosphoinositide Hydrolysis

To determine whether Aldo-induced increases in Ang II receptor number were mediated by decreases in intracellular potassium, cell potassium content was measured after incubation with Aldo. Potassium content in cells exposed to buffer was 1,540±64 nEq/mg protein. Cells exposed to 1 μM Aldo for 40 hours possessed similar potassium contents (99±2% of control, n=3).

To determine if Aldo-induced increases in Ang II binding were consequent to mitogenic influences of Aldo, the effects of Aldo on thymidine incorporation were evaluated. VSMC were incubated with [3H]thymidine and varying concentrations of Aldo for 40 hours. The presence of Aldo did not alter thymidine incorporation: incubation with 0, 10, and 100 nM Aldo resulted in 401±8, 441±12, and 422±18 incorporated cpm, respectively (n=4, no significant differences). These data suggest that increases in Ang II receptor number after incubation with Aldo did not result from stimulation of cell replication.

Despite the fact that Aldo was not mitogenic, the time course for Aldo-induced increases in Ang II receptor number was consistent with new protein synthesis. Therefore, we determined the effects of the protein synthesis inhibitor cycloheximide on Aldo-mediated increases in Ang II binding. We have shown previously that cycloheximide, at concentrations used in this study, inhibited protein synthesis at 37°C in VSMC and did not interfere with the Ang II binding assay. Table 1 shows that incubation with 1 μM Aldo resulted in 63% increase in Ang II binding. Increases in Ang II binding were abolished when 0.5 μM cycloheximide was included with Aldo in the incubation. These results suggest that Aldo-induced increases in Ang II receptor number require the synthesis of new proteins.

Mechanisms of Aldosterone-Induced Increases in Angiotensin II Receptor Number

To determine whether Aldo-induced increases in Ang II receptor number were associated with increases in Ang II receptors, we measured IP levels after incubation with Aldo. Incubation with Aldo for 40 hours was followed by exposure to 100 nM Ang II-stimulated phosphoinositide hydrolysis, we measured IP levels after incubation with Aldo. Incubation with Aldo, alone compared with 15±10% increase with Aldo and spironolactone, n=3). Spironolactone (1 μM) alone did not reduce Ang II binding (106±3% of control, n=3).

TABLE 1. Effects of Cycloheximide on Aldosterone-Induced Increases in Angiotensin II Binding

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Ang II binding (of control)</th>
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<tr>
<td>Aldosterone (1 μM)</td>
<td>163±7</td>
</tr>
<tr>
<td>Aldosterone (1 μM) + cycloheximide (0.5 μM)</td>
<td>105±8</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
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Incubations of confluent vascular smooth muscle cells were performed for 15 hours at 37°C in medium containing 0.1% serum. After washing, single concentration angiotensin II (Ang II) binding was performed. Binding with cycloheximide alone was 106±4% of control. n=4. Statistical analysis was by unpaired t test.

Increases in Ang II binding by 67% (40±9%) increase with Aldo alone compared with 15±10% increase with Aldo and spironolactone, n=3). Spironolactone (1 μM) alone did not reduce Ang II binding (106±3% of control, n=3).

To determine whether Aldo-induced increases in Ang II receptor number were associated with increases in Ang II receptors, we measured IP levels after incubation with Aldo. Incubation with Aldo 40 hours was followed by exposure to 100 nM Ang II-stimulated phosphoinositide hydrolysis, we measured IP levels after incubation with Aldo. Incubation with Aldo for 40 hours was followed by exposure to 100 nM Ang II-stimulated phosphoinositide hydrolysis, we measured IP levels after incubation with Aldo. Incubation with Aldo for 40 hours was followed by exposure to 100 nM Ang II-stimulated phosphoinositide hydrolysis, we measured IP levels after incubation with Aldo. Incubation with Aldo for 40 hours was followed by exposure to 100 nM Ang II-stimulated phosphoinositide hydrolysis, we measured IP levels after incubation with Aldo. Incubation with Aldo for 40 hours was followed by exposure to 100 nM Ang II-stimulated phosphoinositide hydrolysis, we measured IP levels after incubation with Aldo.
FIGURE 3. Bar graph shows effects of aldosterone (Aldo) on angiotensin II (Ang II)–induced reductions in Ang II binding. Cells were incubated for 15 hours at 37°C in growth medium with the concentrations of Aldo and unlabeled Ang II shown in the figure. After two washes with saline, acid washing was performed at 4°C for 10 minutes. Then the cells were washed free of the acid solution, and Ang II binding at a single concentration of $^{125}$I-Ang II was performed (n=6). Data are expressed as binding in cells incubated with Ang II or Aldo, or both, compared with binding in cells incubated without hormones. Binding in cells not exposed to either hormone is represented by the dashed line. *Significant (p<0.05) linear trend analysis by one-way analysis of variance.

FIGURE 4. Bar graph shows effect of aldosterone (Aldo) on angiotensin II (Ang II) desensitization of inositol phosphate (IP) responses. After incorporation with $[^{3}H]$myoinositol for 24 hours, cells were incubated for 15 hours at 37°C with 100 nM Ang II with or without 1 μM Aldo in the continued presence of $[^{3}H]$myoinositol. After two saline washes, acid washing was performed at 21°C for 5 minutes. Then the cells were washed free of the acid solution, and a 30-second incubation with 100 nM Ang II at 21°C was performed on all cells. IP response is defined as the percent increase above basal elicited by 30-second exposure to Ang II. The 100% line represents IP responses in nondesensitized cells (i.e., cells not exposed to Ang II or Aldo during the 15-hour incubation). Statistical analysis is by unpaired t test.

3 shows that incubation with 1 μM Ang II for 15 hours at 37°C resulted in 29% reductions in Ang II surface binding. When 1 nM Aldo was included in the incubation, Ang II–induced reductions in Ang II binding were limited to 14%. When 1 μM Aldo was included in the incubation, Ang II–induced reductions in Ang II binding were no longer observed. In fact, binding was 15% higher than in control cells (cells not incubated with Ang II or Aldo). These data demonstrate that Aldo offset Ang II–induced reductions in Ang II surface receptor number.

Effects of Incubation With Aldosterone on Angiotensin II Desensitization

Exposure of VSMC to Ang II results in desensitization of subsequent Ang II–induced IP responses. This desensitization is mediated, in part, by loss of cell surface receptors. Since Aldo prevented Ang II–induced loss of surface receptors, we questioned whether Aldo could offset Ang II desensitization of IP formation. Figure 4 shows that incubation with Aldo partially reversed Ang II–induced desensitization. Fifteen hours of incubation with 100 nM Ang II desensitized IP responses to subsequent Ang II exposure by 50–60%. When 1 μM Aldo was included in the 15-hour incubation, the magnitude of the desensitization was reduced to 25–30%.

Discussion

In the present studies, incubation of VSMC with Aldo resulted in increases in Ang II binding as a consequence of increases in surface receptor number. These increases were not associated with decreases in cellular potassium, were dependent on new protein synthesis, and were partially prevented by the Aldo receptor antagonist spironolactone. Ang II–stimulated IP formation was increased in proportion to Aldo-induced increases in Ang II receptor number. Finally, Aldo offset Ang II–mediated downregulation of Ang II receptor number and blunted Ang II desensitization of IP formation.

Prior studies using in vivo administration of mineralocorticoids suggest that mineralocorticoids alter Ang II binding. The reported effects of systemic administration of Aldo on Ang II binding to rat cell membranes have varied. Increased receptor number in vascular tissue and decreased receptor number in renal glomerular tissue have been reported. It is difficult to ascertain whether the Aldo effects on Ang II binding were direct since these studies were performed under in vivo conditions and the secondary effects of Aldo on serum potassium and Ang II were not reported. Several studies of mineralocorticoid influence on Ang II binding have been performed in isolated systems. Sumners and Fregly reported that incubation of neuronal cultured cells with deoxycorticosterone acetate resulted in concentration-dependent increases in Ang II surface receptor number. Moreover, in studies by Schiffrin et al. 18-hour incubation of cultured VSMC with Aldo at one concentration (3 ng/ml) elicited 30–50% increases in Ang II binding. Our results expand these earlier observations by demonstrating that Aldo-induced increases in Ang II receptors are time-dependent, concentration-dependent, and are not mediated by changes in cellular potassium content or Ang II concentration.
Our results suggest that Aldo-induced increases in Ang II receptors are mediated through the mineralocorticoid receptor. Increases in binding were significantly inhibited by 100-fold excess of spironolactone. Specific Aldo binding sites have been demonstrated in VSMC. However, since the mineralocorticoid antagonist only partially reversed increases in Ang II binding, Aldo-induced increases in binding may occur through additional pathways, such as glucocorticoid receptors, other steroid receptors, or nonreceptor mechanisms. The latter seems unlikely because of the protracted time course and the requirement for newly synthesized protein.

Although our studies suggest that new protein synthesis is required for Aldo-induced increases in Ang II surface receptors, the identity of this protein (or proteins) is unknown. In epithelial tissue such as toad bladder or renal cortical collecting duct, Aldo-induced proteins may be luminal sodium channels, basolateral sodium pumps, or metabolic activators of existing pumps. It is not clear how synthesis of such proteins in VSMC could result in increased Ang II surface receptors. Alternatively, Aldo-induced proteins in VSMC may be Ang II receptors themselves, anchoring proteins, or cytoplasmic proteins involved in the insertion of existing Ang II receptors into the membrane.

Aldo-induced increases in Ang II receptors were associated with increases in Ang II-stimulated IP₃ formation (Figure 2). Thus, these "new" Ang II receptors appear to be functional. Similarly, Carroll and Goodfriend reported that testosterone-induced increases in Ang II receptors resulted in augmented Ang II-stimulated Aldo production in the adrenal gland. In an earlier study we showed that there are no spare Ang II receptors for phosphoinositide hydrolysis (i.e., reductions in the number of Ang II surface receptors were associated with comparable reductions in Ang II-stimulated IP₃ formation). Figure 5, comprised of data from the present study and the earlier study, displays Ang II-stimulated IP₃ responses as a function of Ang II binding. The highly linear association between Ang II binding (receptor number) and Ang II-mediated IP₃ formation further demonstrates the tight coupling between Ang II receptors and Ang II-mediated phosphoinositide hydrolysis.

The expression of Ang II surface receptors depends on the size of the Ang II receptor pool and its distribution between the cell surface and cell interior. After exposure to Ang II, Ang II receptors are internalized to the cell interior and recycled to the cell surface. The net effect is that the rate of internalization exceeds the rate of externalization so that the number of surface receptors is reduced and downregulation occurs. Our data indicate that Aldo offsets Ang II–induced downregulation of Ang II surface receptors in VSMC (Figure 3). Schiffrin et al reported similar Ang II–Aldo interactions in vivo. Although it is possible that Aldo impaired Ang II–induced internalization of receptors, it is more likely that Ang II acted independently to prevent Ang II–induced downregulation. Evidence for this hypothesis comes from the time course and protein dependency of hormone-induced alterations in Ang II surface receptor number. Ang II downregulation occurs rapidly (within minutes) and is not prevented by protein synthesis inhibition. In contrast, Aldo upregulation requires more than 3 hours and is prevented by protein synthesis inhibition. Thus, it appears that Aldo upregulates and Ang II downregulates Ang II receptors by different mechanisms, the net effect of which is the neutralization of Ang II–induced downregulation by Aldo.

In addition to downregulation, exposure to Ang II results in desensitization of Ang II receptor-mediated IP₃ formation. Figure 4 demonstrates that Aldo partially offsets Ang II–induced desensitization of IP₃ formation. In VSMC, we showed that Ang II desensitization is mediated by both receptor and postreceptor mechanisms. Since incubation with Aldo had no effect on basal IP₃ levels (i.e., did not activate phospholipase C) and since Aldo prevented Ang II–induced loss of Ang II receptors from the cell surface, our data suggest that the major site at which Aldo interfered with Ang II desensitization was at receptor rather than at postreceptor sites.

The present studies suggest that the relation between Ang II and Aldo may be more complex than previously recognized. Increases in Ang II result in increases in Aldo production with subsequent renal sodium retention. Our studies suggest that Aldo directly augments Ang II action in VSMC. These results may be relevant to clinical conditions in which there are excesses of both Ang II and Aldo, such as congestive heart failure and renovascular hypertension. Increases in Ang II alone would be expected to downregulate Ang II receptors and desensitize Ang II-stimulated vascular IP₃ responses. However, concomitant increases in Aldo would offset Ang II–induced downregulation and desensitization. Since IP₃ mediates initial increases in cytosolic calcium and vascular contraction, Aldo-induced increases in Ang II receptors and Ang II–stimulated IP₃ formation may maintain or enhance vascular tone in states of high Ang II and Aldo.

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