

Aldosterone, Not Estradiol, Is the Physiological Agonist for Rapid Increases in cAMP in Vascular Smooth Muscle Cells

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Background—Steroid-induced gene regulation in the endocrine tissues and vascular wall is achieved through the interaction of specific receptor proteins and promoters of target genes. In addition to these delayed steroid actions, rapid effects of steroids have been reported in various tissues that were clearly incompatible with the classic theory of genomic steroid action.

Methods and Results—Because high doses of 17β -estradiol have been shown to modulate intracellular cAMP levels in vascular smooth muscle cells, steroid-induced stimulation of adenylate cyclase stimulation and phosphorylation of cAMP response element binding protein was investigated in porcine coronary artery vascular smooth muscle cells. Aldosterone induces a ≈ 1.5 - to 2.5-fold increase in intracellular cAMP levels ($EC_{50} \approx 0.01$ to 0.1 nmol/L) within 1 minute, whereas 17β -estradiol and hydrocortisone act only at supraphysiological concentrations (10 μ mol/L). Aldosterone-induced changes in intracellular cAMP are calcium dependent; they are not blocked by inhibitors of mineralocorticoid receptors, transcription, or protein synthesis. In addition, aldosterone induces a time-dependent phosphorylation of cAMP response element binding protein with potential transcriptional importance.

Conclusions—A nongenomic modulation of vascular smooth muscle cells by aldosterone is consistent with the data that aldosterone, not estrogen, is the physiological stimulus for cAMP. (*Circulation*. 1999;99:1485-1491.)

Key Words: vasculature ■ pharmacology ■ hormones ■ muscle, smooth

Steroid effects in classic target tissues have been attributed to gene regulation via intracellular receptors. Regulation is achieved through the interaction of specific steroid receptor proteins and promoters of target genes. Mineralocorticoids and glucocorticoids differ markedly with regard to their physiological and clinical effects, although intracellular receptor proteins are closely related and widely promiscuous.^{1,2} Transcription is enhanced via a common, nonspecific consensus DNA sequence, originally designated as glucocorticoid-response element³ or hormone-responsive element. Because both receptor binding and response elements are not sufficient to confer specificity and the complexity of mineralocorticoid or glucocorticoid effects, other mechanisms such as the differential actions of receptors, including nonreceptor factors,⁴ differential metabolism of steroids in hormone responsive tissues as described for 17β -hydroxy-steroid dehydrogenase,^{5,6} or additional nonclassic receptors, may be involved.

The existence of nongenomic effects of steroids such as those on neuronal activity (reviewed in References 7 and 8) or vasoregulation⁹⁻¹³ is supported by expanding experimental evidence and points to the involvement of such nonclassic receptors. Most of these effects can be observed within minutes after steroid exposure¹⁴⁻¹⁶ and are not blocked by inhibitors of protein synthesis, nuclear transcription, or both.

The mechanisms underlying these rapid effects are not completely understood, but they may involve direct action on membrane receptors for steroids.^{17,18} In this context, it is remarkable that specificity, lacking those elements of steroid action as described above, may be found at the level of nongenomic steroid action.^{13,16}

Nongenomic and genomic steroid actions may be interrelated (“cross-talk”). Neuropeptide gene transcription may be promoted by estrogen, although estrogenic response elements^{19,20} are not contained in these genes. The promoters of these genes do, however, contain active cAMP-response elements (CREs) and cross-talk through cAMP signaling is likely to occur. Cross-talk of adrenergic signaling through cAMP and steroid actions is further supported by the data of Nordeen et al²¹ and others,²² who demonstrated a synergistic transactivation of steroid-induced gene expression by the addition of 8-bromo-cAMP.

Although some of these estrogenic effects on cAMP signaling have been found at physiological estrogen concentrations, most of these actions in cardiovascular effector cells have been shown to occur only at high, micromolar concentrations of estrogens.^{23,24} Thus, estrogen might not be the physiological agonist for these processes. Because aldosterone has been shown to rapidly act on intracellular calcium

Received July 23, 1998; revision received November 9, 1998; accepted November 18, 1998.

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and phosphoinositide hydrolysis in vascular smooth muscle cells at physiological concentrations,^{15,25} the aim of the present study was to investigate various steroids, including aldosterone and estrogen, as potential agonists affecting intracellular cAMP levels in porcine coronary vascular smooth muscle cells (PCVSMCs). To follow the sequelae of steroid-induced stimulation of adenylate cyclase, phosphorylation of CRE binding protein (CREB) was tested because it may link cAMP levels to transcription.²⁶

Methods

Materials

Isoproterenol and aldosterone were from Fluka. Cycloheximide, spironolactone, 17 β -estradiol, and hydrocortisone from Sigma. 3-Isobutyl-1-methylxanthine (IBMX) was from BIOMOL. All other chemicals used were of analytical grade and were from Merck AG.

Monoclonal antibodies against the α -smooth muscle isoform of actin and against the smooth muscle isoform of myosin were purchased from Progen Biotechnik and Sigma. Collagenase type I was from Worthington Biochemical. Elastase, penicillin/streptomycin, and amphotericin B were from Boehringer Mannheim AG. Soybean trypsin inhibitor was from Serva/Boehringer Ingelheim. FCS was from cc-pro GmbH.

Isolation and Primary Culture of PCVSMCs

PCVSMCs were prepared enzymatically from pig coronary arteries, with minor modifications as described previously.¹⁵ In brief, porcine hearts were obtained from the local slaughterhouse and transported to the laboratory within 30 minutes. Coronary arteries were dissected aseptically under a microscope and placed in ice-cold PBS supplemented with standard amounts of penicillin (10 U/mL)-streptomycin (10 μ g/mL) and amphotericin B (1 U/mL). After mechanical removal of the endothelial layer of the coronary arteries, the medial layer was stripped off with fine forceps and then underwent enzymatic disaggregation (1 mg/mL collagenase, 0.25 mg/mL elastase, 0.375 mg/mL trypsin inhibitor; 85 minutes at 37°C in HEPES-buffered DMEM). The reaction was terminated with 10% FCS. After centrifugation and washing, disaggregated cells were cultured in nutrient mixture Ham's F-12/DMEM (1:5) supplemented with 10% FCS and antibiotics and antimycotics under standard conditions (37°C, 5% CO₂) in 25-cm² cell culture flasks (Falcon) without the use of extracellular matrices. Plating efficiency was \approx 65% to 80% for primary culture and $>$ 95% for the splitting of passaged cells. After 24 hours, the cultures were washed once to remove nonadherent cells and debris and fed with fresh medium. Culture medium was routinely changed each other day. In experiments, only early passage cells (passages 2 to 6) were used 3 to 4 days after seeding. PCVSMCs showed a typical hill-and-valley phenotype, and $>$ 95% of the cells stained positive with a specific monoclonal antibody against the α -smooth muscle isoform of actin and against the smooth muscle isoform of myosin (data not shown).

Stimulus-Induced Changes of Intracellular cAMP Levels

Experiments were conducted 3 to 4 days after cell splitting at \approx 80% confluence and 24-hour cultivation in serum-free medium. Initially, the incubation medium was exchanged by PSS buffer (135 mmol/L NaCl, 5 mmol/L KCl, 1.8 mmol/L CaCl₂, 0.5 mmol/L MgCl₂, 5.5 mmol/L glucose, 20 mmol/L HEPES, pH 7.4) containing 500 μ mol/L IBMX. After 30 minutes, isoproterenol, steroids, the vehicle with corresponding ethanol concentrations, or a combination, were added, and the reaction was stopped at times indicated by aspiration of the buffer and transfer of the dishes on ice. Incubation of cells with vehicle alone (up to 0.1% ethanol in PSS buffer) did not influence intracellular cAMP levels (data not shown). Inhibitors were added 15 or 60 minutes before cell stimulation as indicated. cAMP was determined with a commercial radioimmunoassay (Am-

ersham) after ethanol extraction. Stock solutions of all steroids (10 mmol/L) were prepared in ethanol and stored in glass vials.

Analysis of Phosphorylation Status of CREB After Cell Stimulation

Steroid- and isoproterenol-induced phosphorylation of the serine-133 residue of CREB was measured via immunoblotting, which is accepted as an indicator of CREB activation. In brief, cells were cultured in standard medium with 10% FCS for 3 days; 24 hours before the experiments, the medium was exchanged, and cells were cultured serum free at \approx 80% confluence. Before the experiments, the incubation medium was changed to PSS buffer containing 500 μ mol/L IBMX. After 30 minutes, various stimuli or controls (vehicle without steroids) were added and the reaction immediately stopped at times indicated by transferring the culture dishes on ice. The medium was aspirated, and cells lysed by adding SDS sample buffer. After scraping the cells off the plate with a rubber policeman, samples were heated, and aliquots were loaded on a 10% SDS-polyacrylamide gel. After electrotransfer to a PVDF-membrane (Amersham), phosphorylated CREB was detected through the binding of specific antibody against the phosphorylated serine-133 residue of CREB (New England Biolabs) and the enhanced chemoluminescence method (Phototype; New England Biolabs). Agonist-induced changes in the phosphorylation status were semiquantitatively determined with densitometry (Image Master ID; Pharmacia). The integral of the absorbance of detected bands (A \times mm) was used in calculations. Relative values of these integrals did not substantially differ from results obtained through calculations of peak absorbances of bands.

Statistical Analysis

Results are presented as mean \pm SEM. Statistical comparisons of different conditions were made with commercial software with the use of nonparametric tests. Friedman's analysis was used for multiple comparisons, and Wilcoxon test was used for single comparisons (StatView SE+ Graphics for Apple MacIntosh); probability value of $<$ 0.05 was considered statistically significant.

Results

Effects of Aldosterone and Isoproterenol on Basal cAMP Levels

The basal cAMP level in PCVSMCs was 1.55 \pm 0.17 pmol/10⁶ cells. Incubation of the cells with aldosterone (100 nmol/L) increased intracellular cAMP levels \approx 1.5- to 2.5-fold within 1 minute versus control conditions (0.0001% ethanol, P $<$ 0.05; Figure 1). Stimulation of PCVSMCs with isoproterenol (10 μ mol/L), an α -adrenoceptor stimulator, induced a time-dependent, \approx 6-fold increase of intracellular cAMP levels (P $<$ 0.05). Aldosterone-induced increases in intracellular cAMP were concentration dependent with cAMP concentrations reaching 234% of control levels at 100 nmol/L (Figure 2). The EC₅₀ value of this effect is between 0.01 and 0.1 nmol/L (Figure 2). Basal cAMP values were not affected by the addition of the vehicle (up to 0.1% ethanol) to the incubation medium.

Because aldosterone-induced effects in rat vascular smooth muscle cells seem to involve calcium signaling,¹⁵ the influence of calcium on cAMP effects was investigated (Table 1): stimulation by aldosterone in calcium-free buffer increased intracellular cAMP concentrations (135% of control values; P $<$ 0.05), but the extent was less than that in controls. Also, after preemptying of inositol-1,4,5-trisphosphate-sensitive stores by the Ca²⁺-ATPase inhibitor thapsigargin (1 μ mol/L), aldosterone-induced effects were small but significant (124%

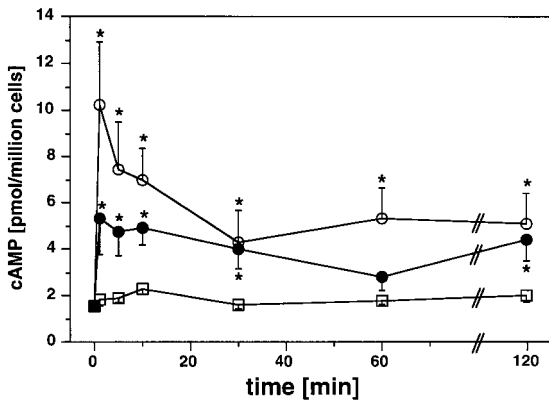


Figure 1. Time course of intracellular cAMP stimulation in PCVSMCs by aldosterone and isoproterenol. Cells were incubated in HEPES-buffered PSS (pH 7.4) containing 500 $\mu\text{mol/L}$ IBMX alone (control, 0.01% ethanol; \square), with aldosterone (100 $\mu\text{mol/L}$; \bullet), or with isoproterenol (10 nmol/L; \circ). Levels of intracellular cAMP were measured with a specific radioimmunoassay (see "Methods"). Values are mean \pm SEM (n=5 for isoproterenol and n=8 for control and aldosterone). * P <0.05 vs control.

of control values; P <0.05). Preincubation of cells in calcium-free buffer with thapsigargin completely abolished aldosterone-induced changes in intracellular cAMP (Table 1; NS).

Steroid Specificity and Inhibitors

Hydrocortisone and 17 β -estradiol significantly increased cAMP only if concentrations as high as 10 $\mu\text{mol/L}$ were used (173% or 139% of control values; P <0.05; Figure 2).

Preincubation of cells with the inhibitors of transcription and protein synthesis for 15 minutes did not block rapid aldosterone-induced effects on intracellular cAMP levels: aldosterone (100 nmol/L) stimulated intracellular cAMP levels by $\approx 154 \pm 12\%$ without inhibitors (P <0.05), by $\approx 217 \pm 21\%$ during preincubation with actinomycin D (5 $\mu\text{g/mL}$), and by $150 \pm 19\%$ during preincubation with cycloheximide (20 $\mu\text{g/mL}$). Similar results were obtained after 60-minute preincubation with inhibitors, which is considered to be a sufficient time period for inhibitors to take effect,^{27,28}

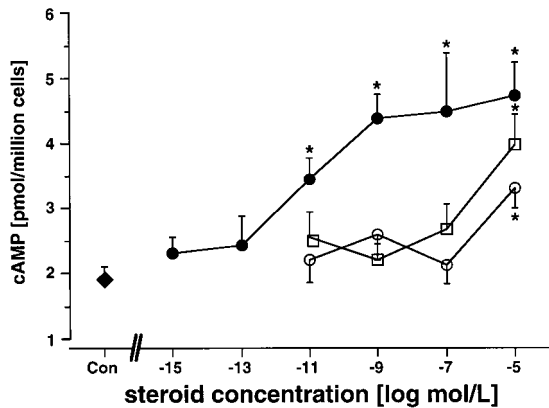


Figure 2. Dose-response curves for aldosterone (\bullet), hydrocortisone (\circ), and 17 β -estradiol (\square) effects on intracellular cAMP levels (pmol/ 10^5 cells) in PCVSMCs after incubation for 1 minute. Incubation conditions were same as in Figure 1 (\blacklozenge , control). Values are mean \pm SEM (n=6). * P <0.05 vs respective baseline values.

Relative Changes in Intracellular cAMP Levels After Stimulation With Aldosterone vs Respective Control (0.01% Ethanol) for 1 Minute

Condition	Aldosterone (100 nmol/L)
PSS (n=14)	154 \pm 12%*
Ca ²⁺ free (n=12)	135 \pm 10%*
Thapsigargin (1 $\mu\text{mol/L}$) (n=8)	124 \pm 5%*
Ca ²⁺ free, thapsigargin (1 $\mu\text{mol/L}$) (n=10)	93 \pm 8%

Before cell stimulation, cells were preincubated for 10 to 15 minutes in modified PSS buffer as indicated (mean \pm SEM).

* P <0.05 vs control without aldosterone.

and subsequent stimulation of cells with 10 nmol/L aldosterone for 1 minute (Figure 3). Incubation of PCVSMCs with inhibitors alone did not significantly influence basal cAMP levels.

In addition, 15-minute incubation of cells with spironolactone (10 $\mu\text{mol/L}$), a classic antagonist of genomic mineralocorticoid action, did not significantly blunt aldosterone-induced effects on intracellular cAMP levels (Figure 4; 186% versus 162% of control values). Similar results were obtained after a 60-minute preincubation of the cells in spironolactone (10 $\mu\text{mol/L}$): aldosterone (10 nmol/L) significantly increased intracellular cAMP levels from 2.58 ± 0.10 to 4.85 ± 0.37 pmol/ 10^5 cells without (n=8; P <0.05) and from 2.48 ± 0.20 to 4.89 ± 0.60 pmol/ 10^5 cells (n=6; P <0.05) with the continued presence of spironolactone. Basal cAMP levels were not significantly influenced by the preincubation of cells in spironolactone for 60 minutes.

Phosphorylation of CREB

Stimulation of cells with isoproterenol and aldosterone increased immunodetectable phosphorylation of CREB within minutes, whereas the addition of the solvent alone did not significantly influence CREB phosphorylation

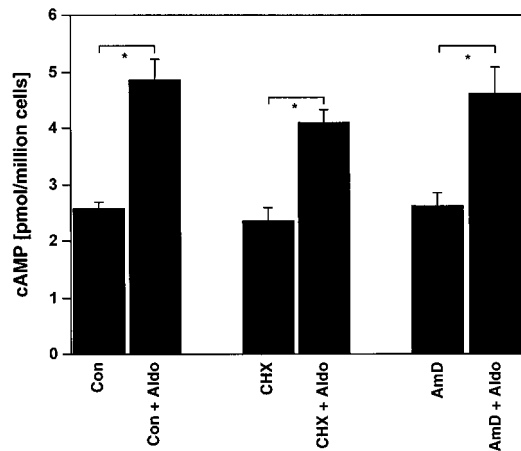


Figure 3. Effects of inhibitors of transcription and protein synthesis on rapid aldosterone effects in PCVSMCs, which were incubated with cycloheximide (20 $\mu\text{g/mL}$; CHX), actinomycin D (5 $\mu\text{g/mL}$; AmD), or control (ethanol 0.1%; Con) in HEPES-buffered PSS containing 500 $\mu\text{mol/L}$ IBMX for 60 minutes. This incubation was followed by addition of aldosterone (10 nmol/L) or vehicle for 1 minute. Values are mean \pm SEM (n=6 to 8). * P <0.05.

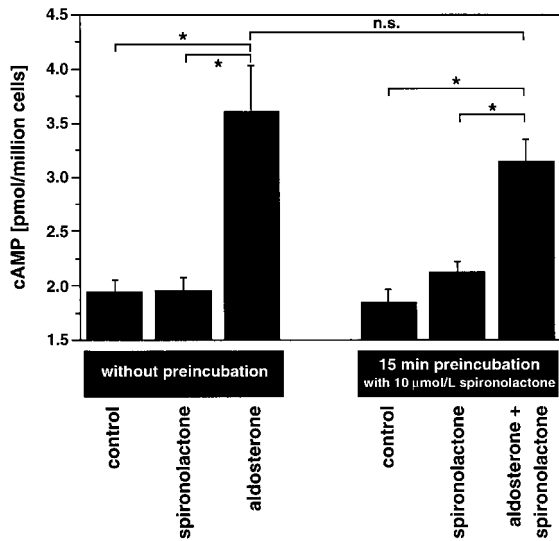


Figure 4. Effect of spironolactone on rapid aldosterone effects in PCVSMCs. Cells were incubated with vehicle (control, 0.01% ethanol) or spironolactone (10 μ mol/L) with or without aldosterone (100 nmol/L) in HEPES-buffered PSS containing 500 μ mol/L IBMX for 1 minute after a 15-minute preincubation without (left) or with (right) spironolactone (10 μ mol/L). Values are mean \pm SEM (n=6 to 8). * P <0.05.

(Figure 5). Levels of phosphorylation significantly increased to $128 \pm 16\%$ (n=10; P <0.05) and $150 \pm 23\%$ (n=6; P <0.05) after stimulation of cells with 1 and 100 nmol/L aldosterone for 10 minutes, respectively, whereas 10 μ mol/L isoproterenol increased phosphorylation levels of CREB to $409 \pm 125\%$ of control levels (n=6; P <0.05). Differences in phosphorylation levels correlate with differences in the increases of intracellular cAMP after stimulation with aldosterone or isoproterenol. Stimulation of cells with 0.1 μ mol/L estradiol did not significantly change levels of CREB phosphorylation ($111 \pm 17\%$ versus control; n=6; NS), whereas the stimulation with 10 μ mol/L estradiol significantly induced CREB phosphorylation within 10 minutes ($174 \pm 61\%$ versus control; n=6; P <0.05). A similar concentration-dependent correlation between estradiol-induced increases in cAMP and CREB phosphorylation was found for hydrocortisone, whereas 0.1 μ mol/L hydrocortisone was ineffective ($111 \pm 7\%$ versus respective control; n=7; NS), and 10 μ mol/L hydrocortisone significantly increased levels of CREB phosphorylation versus control ($147 \pm 28\%$ versus control; n=7; P <0.05). Coincubation of aldosterone with increasing concentrations of isoproterenol was performed to search for synergistic effects. After preincubation of cells with 100 nmol/L aldosterone for 10 minutes, isoproterenol increased phosphorylation of CREB at \approx 10-fold lower concentrations to levels obtained in experiments without aldosterone, suggesting an at least additive, if not synergistic, action of isoproterenol and aldosterone (Figure 6). Comparable sensitization of isoproterenol (0.1 nmol/L for 5 minutes)-induced increases in pCREB levels were found during coincubation with 10 μ mol/L estradiol or hydrocortisone ($180 \pm 42\%$ and $146 \pm 23\%$ versus control; n=7; P <0.05), whereas 0.1 nmol/L isoproterenol alone

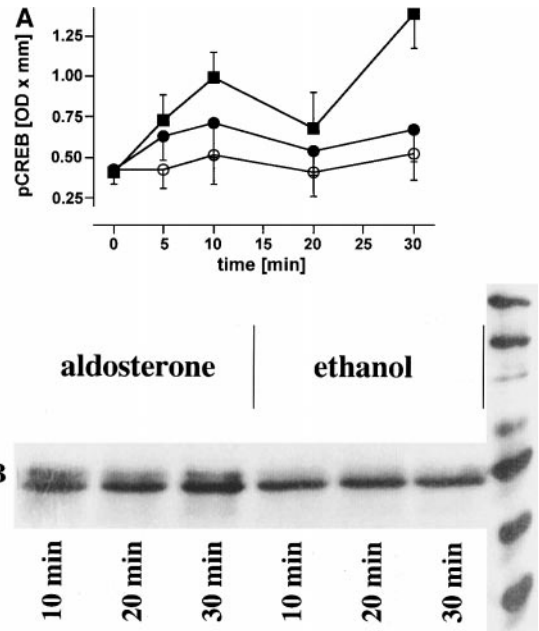


Figure 5. A, Effects of aldosterone or isoproterenol on phosphorylation of CREB. Cells were incubated in PSS buffer alone (control, 0.01% ethanol; \circ), with aldosterone (100 nmol/L; \bullet), or with isoproterenol (10 nmol/L; \blacksquare) for times indicated. After incubation, cell lysates were separated on 12% SDS-PAGE, and phosphorylated CREB was detected immunologically after transfer on PVDF membranes (see "Methods"). Bands were measured densitometrically, and data are expressed as trace values (absorbance \times mm). Values are mean \pm SEM (n=4). B, Representative immunoblot demonstrating time-dependent effects of aldosterone on phosphorylation of CREB (pCREB). Cells were incubated in PSS buffer alone or with aldosterone for times indicated. Incubation conditions and methods were same as described in A.

did not significantly change CREB phosphorylation ($114 \pm 8\%$; n=21; NS). However, aldosterone induced an increase in CREB phosphorylation during coincubation with 0.1 nmol/L isoproterenol, even at a concentration as low as 1 nmol/L ($199 \pm 0.5\%$; n=11; P <0.05).

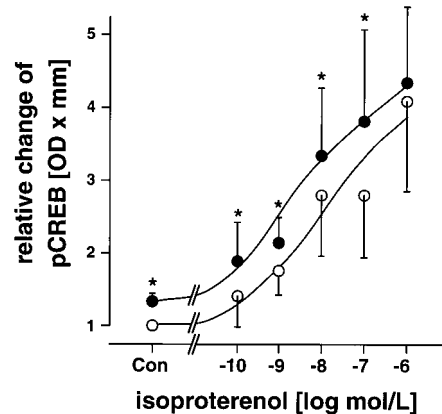


Figure 6. Effects of aldosterone on isoproterenol-induced phosphorylation of CREB. Cells were incubated in PSS buffer alone (control: 0.01% ethanol; \circ) or after preincubation for 5 minutes with aldosterone (100 nmol/L; \bullet). Increasing concentrations of isoproterenol (0.1 nmol/L to 1 μ mol/L) were added for 5 minutes (incubation conditions are same as in Figure 5). Bands were measured densitometrically, and values are expressed relative to baseline values without aldosterone (mean \pm SEM, n=6). * P <0.05 vs control without aldosterone; curves are drawn by hand.

Discussion

The main findings of the present study are that (1) aldosterone specifically increases intracellular levels of cAMP in PCVSMCs within minutes, and the effect is not blocked by inhibitors of transcription and protein synthesis; (2) the EC₅₀ value of ≈ 0.01 to 0.1 nmol/L is close to the free physiological plasma concentration of aldosterone in humans²⁹; (3) hydrocortisone and 17 β -estradiol are active only at supraphysiological concentrations; (4) CREB is rapidly phosphorylated in response to aldosterone but not in response to estradiol or hydrocortisone at comparable concentrations; and (5) isoproterenol effects occur at lower concentrations after pretreatment with aldosterone.

These effects are in line with the effects of aldosterone in vascular smooth muscle cells described previously for Ca²⁺, inositol-1,4,5-trisphosphate, and protein kinase C.^{15,16,25} They are classified as nongenomic effects because they are rapid and not inhibited by the mineralocorticoid receptor antagonist spironolactone or by inhibitors of transcription and protein synthesis. A new pathway through specific membrane receptors for aldosterone was previously suggested^{12,17,30} to transmit these rapid aldosterone actions. The receptors involved are clearly distinct from the cytosolic type 1 mineralocorticoid receptor,³¹ which does not distinguish mineralocorticoids from glucocorticoids. Radioactive binding studies in membrane preparations from human mononuclear leukocytes, porcine kidney, and porcine liver^{17,30,32} suggest a binding site compatible with major features of nongenomic aldosterone action (eg, specificity, low binding of spironolactone).

The interaction of steroids with specific receptors is not the only mechanism of rapid action known to date: steroid effects at supraphysiological concentrations may be induced by nonspecific membrane interactions.^{33,34} However, rapid aldosterone effects on cell signaling, including intracellular cAMP, CREB phosphorylation, calcium,¹⁶ and phosphoinositide hydrolysis,^{15,25} occur at subnanomolar physiological concentrations of aldosterone, suggesting the involvement of specific mechanisms. In contrast to rapid aldosterone actions, rapid estradiol-induced increases of cAMP in vascular smooth muscle cells of rat pulmonary artery,²³ effects on calcium currents of vascular smooth muscle cells,³⁵ and regulation of vascular tone predominantly occur at micromolar concentrations,^{24,36} whereas the rapid actions at physiological estradiol concentrations have been demonstrated only in cells of fetal origin^{37–39} or undifferentiated, multipotent cells.⁴⁰ Because supramicromolar concentrations of estrogens are nonphysiological, perhaps even nonpharmacological, the data presented here suggest that aldosterone, but not 17 β -estradiol, is the physiological stimulus of cAMP in vascular smooth muscle cells of porcine coronary arteries.

With regard to the intracellular signaling leading to cAMP stimulation, it should be noted that free intracellular calcium has been shown to be involved in nongenomic action of aldosterone in PCVSMCs at low physiological concentrations.¹⁶ Preempting of inositol-1,4,5-trisphosphate-sensitive stores by thapsigargin in calcium-free buffer abolished rapid effects on intracellular cAMP.

These data are in line with those demonstrating dependence of cAMP-mediated contractility on modulation of intracellular calcium signaling.⁴¹ Thus, a calcium/calmodulin-dependent adenylate cyclase may be involved in aldosterone-induced effects on intracellular cAMP levels as suggested by Zhang et al.⁴²

Increases in intracellular cAMP after stimulation with aldosterone are markedly lower than increases after stimulation with isoproterenol or forskolin. Given this, it is even more important to review evidence supporting the physiological relevance of rapid aldosterone effects. Suppression of baroreceptor discharge has been demonstrated in a canine model within 15 minutes of aldosterone injection.⁴³ Rapid reduction of coronary flow and increases of aortic flow and cardiac output were found in an isolated rat working heart model.⁴⁴ Klein and Henk⁴⁵ showed rapid aldosterone effects on systemic vascular resistance in humans occurring within 5 minutes. Furthermore, in a placebo-controlled, randomized trial, rapid aldosterone-induced increases in phosphocreatine levels have been shown for calf muscle during recovery from submaximal exercise.⁴⁶ Although the *in vivo* and *in vitro* effects of aldosterone alone appear small, its synergisms with other cardiovascular hormones may be physiologically important. An at least additive, if not synergistic, effect is suggested by data on the combined aldosterone/isoproterenol action shown here and by earlier data on angiotensin II/aldosterone effects on [Ca²⁺]_i.¹⁶ Another important issue concerning the physiological relevance of rapid aldosterone effects involves a comparison of aldosterone concentrations required for rapid *in vitro* action with those present *in vivo*. Aldosterone levels producing half-maximal effects (0.01 to 0.1 nmol/L) *in vitro* are relatively low in terms of the normal range of circulating aldosterone plasma concentrations (0.1 nmol/L in humans²⁹). Therefore, a tonic modest stimulation of the system may be present under physiological conditions. Lowering of aldosterone levels may be a protective mechanism in, for example, salt loading, by a possibly changed sensitivity to catecholamine effects. This appears to be even more relevant as the concentrations required *in vitro* are commonly higher than those required *in vivo*.

In addition to these direct or indirect rapid cardiovascular effects, nongenomic effects of aldosterone on intracellular signaling^{15,16,25} may modulate long-term actions of aldosterone or other steroids on the cardiovascular system through the activation of transcriptional coactivators such as CREB. This assumption is in line with data suggesting a modulation of steroid-induced gene transactivation by increases in intracellular cAMP: modulation of transcription rates after stimulation with glucocorticoids or mineralocorticoids has been demonstrated in cells transfected with glucocorticoid²¹ or mineralocorticoid receptors²² during coincubation with 8-bromo-cAMP. Thus, modulation of intracellular signaling may be involved in the determination of specificity of mineralocorticoid and glucocorticoid actions,²² as suggested recently.^{12,13}

In summary, aldosterone rapidly increases intracellular cAMP levels within minutes at subnanomolar concentrations, whereas both estrogen and hydrocortisone are active only at

supramicromolar concentrations. Phosphorylation of CREB by aldosterone, as a known coactivator of genomic steroid action, suggests that aldosterone may influence transcription through its rapid action on phosphorylation mechanisms. Because the half-maximal effects of aldosterone are seen at concentrations close to or even below free circulating plasma aldosterone levels, a tonic modest stimulation of this system may be the normal physiological state. Thus, changes in aldosterone plasma levels during alterations of body water or electrolyte balance may be involved in the fine tuning of vasoregulation via modulation of sensitivity to other vaso-regulators such as catecholamines or angiotensin II. The development of inhibitors that block both rapid nongenomic and subsequent genomic steroid effects may be relevant in future drug developments for the treatment of important cardiovascular diseases, such as hypertension and chronic heart failure.

Acknowledgments

This study was supported by a grant of the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (No. 01EC9407). We thank Elke Kirsch for expert technical assistance. Some results presented in this study are part of the doctoral thesis of Andreas Günther at the Faculty of Clinical Medicine Mannheim, University of Heidelberg.

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