Angiotensin-Induced Desensitization of the Phosphoinositide Pathway in Cardiac Cells Occurs at the Level of the Receptor

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Previous studies show that angiotensin II (Ang II) increases phosphoinositide turnover in cultured neonatal heart cells. Ang II has also been shown to transiently increase spontaneous beating behavior in these cells. In this study we seek to identify the molecular mechanism underlying this rapid (3–5-minute) desensitization. Time-course studies on the accumulation of [3H]inositol phosphates indicate that the loss in functional responsiveness correlates with reduced efficacy of Ang II to activate the phosphoinositide path. Binding studies with 125I-Ang II revealed that there was no change in surface receptor binding capacity during the time in which desensitization developed. Normal phosphoinositide and functional responses are observed when desensitized cells are treated with probes that activate the cardiac phosphoinositide pathway at discrete steps. These studies reveal that the functional status of the major components of the phosphoinositide signaling pathway, including G proteins, phospholipase C, and protein kinase C (PKC), are normal during maintained Ang II desensitization. To study the potential role of PKC in Ang II desensitization, the cells are treated with TPA for 24 hours, which downregulates PKC activity. PKC-depleted cells rapidly desensitize after Ang II application. We conclude that the selective Ang II–evoked biochemical/functional desensitization involves inhibition at the level of the receptor, rather than at a component downstream in the path, and that this process is independent of PKC and loss of surface binding capacity. (Circulation Research 1991;69:800–809)

Desensitization, or loss in responsiveness, is an adaptive process displayed by many cells when exposed to a variety of external stimuli or agonists. Agonist-induced desensitization represents an important regulatory mechanism in many tissues. For example, persistent activation of the β-receptor in cardiac muscle results in a desensitization of the cAMP response.1 This change in the cardiac responsiveness significantly limits the opportunity to pharmacologically regulate cAMP in desensitized heart tissue. Although the molecular mechanisms underlying desensitization of many agonist-activated signal transduction systems are poorly understood, specific pathways have been identified in the uncoupling of the β2-adrenergic–adenylate cyclase system.2,3 In this system, alterations in the receptor rather than postreceptor mechanisms are involved. Receptors for the Ca2+-mobilizing hormone, angiotensin II (Ang II),1 are coupled to the phospholipase C/phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2] signaling system in many target tissues, including vascular smooth muscle as well as heart.4–6 Agonist binding stimulates phospholipase C, which leads to hydrolysis of PtdIns(4,5)P2, generating two second messengers, inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol, the latter of which activates protein kinase C.7 There are reports8,9 demonstrating that this Ang II receptor-effector system can rapidly desensitize, within minutes, after exposure to agonist. Receptor internalization may underlie the desensitization responses of a number of hormones. In this regard, translocation of the Ang II receptor from the cell surface has been observed in response to application of agonist.9,10 Several reports8,11,12 suggest that protein kinase C may be an important inhibitory regulator of the Ang II–induced activation of phospholipase C in vascular smooth muscle, renal, and epithelial cells. Thus, in a number of target tissues, potential mechanisms of Ang II desensitization have been documented.

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The basis for the present study arises from our initial results indicating that persistent stimulation of Ang II receptors leads to a functional desensitization of cardiac cells. The focus of this study was to determine if the underlying molecular mechanism of this desensitization involved Ang II receptors or whether postreceptor components were altered.

Materials and Methods

Materials

The following substances were obtained from Sigma Chemical Co., St. Louis, Mo.: Dulbecco’s modified Eagle’s medium (DMEM), DMEM-HEPES, formic acid, ammonium formate, phenylephrine, and 12-O-tetradecanoylphorbol 13-acetate (TPA). Perchloric acid was from J.T. Baker Chemical Co., Phillipsburg, N.J. Fetal calf serum was purchased from Inovar Inc., Gaithersburg, Md. Ang II was from Peninsula Laboratories, Inc., Belmont, Calif. [3H]Inositol, 124-1-Ang II, and [3H]IP3 were from NEN Research Products, Wilmington, Del., and Dowex AG1-X8 (formate form) was from Bio-Rad, Rockville Centre, N.Y. All other agents were purchased as reagent grade.

Tissue Culture

Primary cultures of neonatal rat cardiac ventricular myocytes were prepared from 1-day-old Sprague-Dawley rats as previously described. After dissociation of the tissue, the cells were seeded in four-well plastic tissue culture dishes (1.7-cm wells) with growth medium (DMEM, 10% fetal calf serum, 1% penicillin, and 1% streptomycin). The cells were maintained in a humidified atmosphere of 92.5% air–7.5% CO2.

Measurement of Contractile Properties

The contraction of a single cell in a spontaneously beating multicellular network was measured using an optical–video motion detection system as previously described. Briefly, the four-well culture dish was mounted on the stage of an inverted phase microscope, and the cells in a single well were superfused at 1 ml/min with DMEM-HEPES plus any additional agents as indicated in “Results.” The superfusion medium was equilibrated with 95% O2–5% CO2 and maintained at 32°C. The image of the beating cells was monitored using a video camera (Cohu, San Diego, Calif.), and the video signal was recorded on tape. The motion of the cell wall was measured with the use of a video dimension analyzer (Instrumentation for Physiology & Medicine, Inc., San Diego, Calif.). The video dimension analyzer provides an analog output proportional to the position of the edge of the moving cell. The contraction and relaxation velocities were obtained by electronically differentiating the analog position versus the time output of the video dimension analyzer as described previously. TPA was added to the superfusion buffers from stock solutions in dimethyl sulfoxide, such that the medium contained a final dimethyl sulfoxide concentration of 0.1%. Control experiments indicated that this concentration of vehicle had no effect on contractile behavior.

Measurement of Inositol Phosphates

Cells in culture (4–5 days old) were labeled with 10 µCi/ml [3H]inositol for 48 hours in serum-free DMEM-HEPES, 0.25% bovine serum albumin, unless otherwise indicated. Pilot studies revealed that the absence of serum during the labeling phase had no qualitative effects on the biochemical or biological responses reported in “Results.” However, serum-free medium greatly improved the signal in the assay. At the time of the experiment, the cells were washed with 4×0.5 ml DMEM-HEPES and treated as indicated in “Results.” The reaction was stopped by aspirating the reaction solution and rapidly adding 0.5 ml of 6% perchloric acid at 0°C. The acid extract was neutralized, and the [3H]inositol phosphates were resolved using ion-exchange chromatography as described previously. Total [3H]-inositol phosphates were collected by eluting them from the columns with 3 ml of 2 M ammonium formate/0.1 M formic acid. In several experiments [3H]inositol 1,4-bisphosphate ([3H]IP2) accumulation was measured instead of total [3H]inositol phosphates to achieve improved signal-to-noise ratios. Pilot experiments demonstrated that the accumulation of total [3H]inositol phosphates was linear with respect to time for phenylephrine and the G protein activator, AlF4-, over a range of 30 and 40 minutes, respectively. In all of the experiments, measurements were made in this linear range.

Measurement of Exogenous [3H]IP3 Degradation

Cells that were 5–7 days old were incubated overnight in serum-free DMEM-HEPES. At the time of the experiment, the medium was aspirated, and the cells were washed with 0.5 ml of 60 mM HEPES, 1 mM EGTA, 1 mM EDTA, and 25 µg/ml leupeptin. Cells were then scraped and homogenized in a Teflon/glass homogenizer in the same buffer. The reaction was started by the addition of 0.6 mM [3H]IP3, (20 Ci/mmol) and Mg2+ (to a final concentration of 2 mM) to the cell homogenate in a total volume of 250 µl. The reaction was stopped by the addition of 0.5 ml of 6% perchloric acid and the [3H]-labeled inositol phosphates separated as described above. The assay was linear with respect to protein in the range of 5–10 µg. The IP3 5’-phosphomonoesterase activity was inhibited by 10 mM 2,3-diphosphoglycerate, was dependent on MgCl2, and was found mainly in the particulate fraction. This finding is in agreement with previously published properties for this enzyme.

Measurement of Ang II Surface Receptor Binding Capacity

Previous reports from our laboratory have identified specific, functional high-affinity receptors (in the subnanomolar range) for Ang II on cultured cardiac cells. In the present study, an assay was adapted from
previously described methods to examine Ang II surface binding capacity in intact cells in a time frame consistent with the desensitization responses.\(^9\) Cells, cultured in 1.7-cm multwell plates, were incubated with or without 100 nM Ang II or 100 nM TPA for 5 or 10 minutes, respectively. The effects of these functional desensitization treatments on surface receptor binding capacity were assessed in the following manner. After these treatments, cells were washed once with 0.3 ml of ice-cold 50 mM glycine and 150 mM NaCl, pH 3.0, and were incubated in the same solution for 10 minutes at 4°C. Pilot experiments, in which cardiac cultures were incubated with \(^{125}\text{I-Ang II, revealed that }\geq 95\% \text{ of the specific binding was removed by this acid wash, consistent with what has been observed in other Ang II receptor systems.}\(^9\)\(^10\) Other control experiments indicated that this acid wash had no effect on the Ang II binding capacity of the cultures. At the end of the wash the cells were rinsed with 4 x 0.3 ml binding buffer that consisted of 50 mM Tris, 120 mM NaCl, 4 mM KCl, 1 mM CaCl\(_2\), 0.1 mM bacitracin, and 0.25% bovine serum albumin, pH 7.0. The cells were then incubated with \(\sim 0.1 \text{ nM } ^{125}\text{I-Ang II (2,200 Ci/mmol) in 0.3 ml of the binding buffer for 1 hour at 4°C. Time-course studies revealed that equilibrium was achieved under these binding conditions. The binding reaction was terminated by washing the cells with 4 x 0.3 ml ice-cold binding buffer. The cells were solubilized with 0.5 ml of 0.25N NaOH and 0.5% sodium dodecyl sulfate, and the extracts were counted in a gamma counter (LKB Rack-gamma counter). The results were reported as specific \(^{125}\text{I-Ang II binding (\sim 4,400 cpm/mg protein), which was the difference between the total binding and the nonspecific binding. The nonspecific binding was estimated in parallel binding experiments in which the cells were incubated with 100 nM Ang II along with \(^{125}\text{I-Ang II. The nonspecific binding was in the range of 20–25\% of the total binding in all of the experiments.}

Results

We\(^5\) have previously reported that Ang II rapidly (within 1 minute) increased beating frequency and decreased twitch amplitude in spontaneously beating neonatal myocytes by a receptor-mediated mechanism. As shown in Figure 1, when the cells were exposed to the hormone for longer periods, the contractile behavior returned to control levels within 6 minutes after the initial response. This apparent functional desensitization was not due to degradation of the peptide, since the cells were constantly superfused with Ang II-containing buffer in these experiments.

Previous results\(^5\)\(^-\)\(^15\) have supported the idea that the effects of Ang II are mediated by protein kinase C in cultured heart cells. Ang II and phorbol esters evoked the same changes in contractile behavior and whole-cell Ca\(^{2+}\) current.\(^15\) Further, both agents increased the phosphorylation state of the same set of proteins to the same extent, including an 80-kDa acidic protein, shown to be a substrate for protein kinase C.\(^15\) Since activation of protein kinase C by external stimuli may subsequently result in its inactivation,\(^7\) Ang II–evoked desensitization may involve such a process. However, as shown in Figure 1, in contrast to the effects of Ang II, the functional responses evoked by phorbol esters were sustained for at least 15–20 minutes. These results suggested that the apparent Ang II–evoked desensitization occurred at a step upstream from protein kinase C in the signaling pathway. Further evidence for this hypothesis is presented in Figure 1C, in which TPA still stimulated contractile behavior in cells that were desensitized to Ang II. The results from seven experiments with Ang II–desensitized cells after a 10-minute exposure to 80 nM TPA were as follows: increase in frequency, 69±12%; decrease in ampli-
tude, 56±6% (mean±SEM, n=7). These results were identical to those we5 have previously reported for cultures that have not been desensitized to Ang II. Protein kinase C can still be stimulated in Ang II–desensitized cells.

The kinetics of Ang II–evoked phosphoinositide turnover were characterized to determine if desensitization could be assessed at the biochemical level. As shown in Figure 2, Ang II stimulated the accumulation of IP3, IP2, and inositol 1-monophosphate (IP1) in [3H]inositol-prelabeled cells. The response was rapid; IP2 and IP3 increased 4.5-fold and 2.28-fold, respectively, within 15–30 seconds, after which time the levels returned toward basal values within 3–4 minutes. Since the time course for the functional desensitization is comparable to that for phosphoinositide turnover, it is possible that an uncoupling of the receptor-effector signaling pathway has occurred. A number of tests were performed to probe this hypothesis. Previous reports16 indicated that activation of protein kinase C can lead to increases in the degradation of inositol phosphates. Thus, it is possible that there was still considerable flux through the Ang II signaling path at 4 minutes but that increases in the degradation of IP3, resulting from activation of endogenous phosphatase activity, would have given rise to the observed accumulation time course depicted in Figure 2. To test this hypothesis, we measured the rate of breakdown of [3H]IP3, when added directly to homogenates of control and Ang II–treated cells. As shown in Figure 3, the rate of degradation of [3H]IP3 and the rate of formation of [3H]IP2 and [3H]IP1 in cardiac cell homogenates were not altered by hormone treatment.

Since the masses of inositol phosphates were not measured in these experiments, another explanation for the results in Figure 2 is that the level of the 3H-labeled lipid precursor, phosphatidylinositol, is limiting at 4 minutes. Phenylephrine was used as a probe to explore this possibility, since it had been previously reported that such α-adrenergic agonists also stimulated phosphoinositide hydrolysis in heart cells. 17 As shown in the time-course curve in Figure 4, phenylephrine stimulated phosphoinositide turnover in cultured myocytes with no apparent desensitization. In clear contrast to the pattern of Ang II–evoked stimulation of IP3, the α-agonist-evoked rate of increase was linear for at least 10 minutes, and the stimulation was much greater, eightfold compared with threefold for Ang II. These results supported the idea that the level of hormone-sensitive 3H-labeled phosphatidylinositol is not a limiting factor in these cells.

One potential mechanism of the observed desensitization would be a decrease in surface Ang II receptor number. If this were an important mechanism in the desensitization, then inhibitors of receptor sequestration should prevent desensitization. Phenylarsine oxide and concanavalin A have been reported to block receptor internalization in a num-
The phosphoinositide response in such cultures was found to desensitize to Ang II as control cells (data not shown). This suggested that receptor sequestration was not involved. This hypothesis was explored in more direct experiments. We have previously demonstrated that cultured myocytes express specific, high-affinity Ang II receptors. An assay was adapted from a previously reported method to examine the effects of short-term Ang II treatment on surface receptor binding in intact cells. Cells were exposed to unlabeled 100 nM Ang II, 100 nM TPA (a control for later experiments), or medium at room temperature for 10 minutes. The cells were cooled to 4°C and washed with pH 3 buffer for 10 minutes to remove bound ligand. Then Ang II binding was performed at 4°C for 1 hour and the amount of specific surface binding was quantitated. The values of specific binding under these conditions were as follows (cpm/mg protein × 10^(-3)): control, 4.40 ± 0.49; Ang II-treated cells, 4.04 ± 0.60; TPA-treated cells, 4.61 ± 0.64 (mean ± SEM, n = 4). Taken together, these data do not support a mechanism in which receptor internalization is an important process in a rapid desensitization evoked by Ang II.

To design a useful experimental strategy to further define the mechanism of the loss in Ang II effectiveness, it was essential to know if the desensitization was reversible. Ang II was applied to the cells and then removed by washing. After various time intervals, Ang II was reapplied, and the accumulation of [3H]inositol phosphates was quantitated. As shown in Figure 5, Ang II–desensitized cells remained unresponsive to hormone for 30 minutes after removal of Ang II. After this time, there was a rapid return in the responsiveness, and by 50 minutes, the cells exhibited normal sensitivity. Thus, it was feasible to present Ang II–desensitized cells with various hormones or probes and to measure subsequent [3H]-inositol phosphate accumulation over a 30-minute interval. As shown in Figure 6, desensitized cells displayed a normal phosphoinositide response when stimulated with either phenylephrine or AlF_4^-. Carbachol-stimulated phosphoinositide hydrolysis is also normal in such cells (data not shown). Thus, desensitization that followed Ang II application was confined to the Ang II–phosphoinositide pathway.

The observations that the responses of Ang II–desensitized cells to phenylephrine and AlF_4^- were normal suggested that the site of desensitization occurs upstream from either a G protein or phospholipase C. This hypothesis leads to the prediction that the contractile responses of Ang II–desensitized cells to α1-adrenergic stimulation should be normal. Consistent with other reports, when myocytes were exposed to 5 nM phenylephrine, there was an increase in spontaneous beating rate and a decrease in amplitude (Figure 7A). Consistent with the biochemical responses in Figure 4, the functional responses did not desensitize to continued α-adrenergic stimulation. As shown in Figure 7B, Ang II–desensitized cells responded as control cells when challenged with
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II–desensitized cells, 21±3.6 (n=5). Taken together, results obtained by the use of agents that act at discrete steps in the phosphoinositide pathway support the view that the control of Ang II desensitization occurs at a step upstream from protein kinase C, phospholipase C, and G proteins.

A tentative hypothesis was that protein kinase C mediates Ang II–evoked desensitization, since this enzyme has been reported to play a role in agonist-evoked desensitization of the phosphoinositide pathway in a number of other systems.11,19,20 To test this hypothesis, the Ang II intracellular signaling path was shunted by directly activating protein kinase C with the phorbol ester TPA. TPA treatment (100 nM, 10 minutes) has been shown to evoke contractile responses, increase protein phosphorylation,15 and lead to a translocation of protein kinase C activity from the cytosol to the particulate fraction of cultured myocytes (data not shown). As shown in Figure 8, TPA treatment mimicked the effects of Ang II in desensitizing the cells to the peptide. Although these data suggested that protein kinase C is an important mediator of the Ang II–evoked desensitization of myocardial cells, further studies demonstrated important differences between the Ang II and the TPA effects. In particular, TPA treatment resulted in cells that were not only desensitized to Ang II, but were also desensitized to phenylephrine stimulation (Figure 8). The phosphoinositide response observed by direct G protein activation with AlF₄⁻ was not altered by TPA (Figure 8). This last result suggested that the action of TPA was like Ang II in that it altered a step in the signaling path that precedes G proteins. However, protein kinase C activation evoked a more complex pattern of desensitization compared with the peptide.

A likely hypothesis from the results in Figure 8 was that the Ang II mechanism involves molecular pro-

FIGURE 7. Effects of angiotensin II (AII) desensitization on functional responses of myocytes to phenylephrine. Beating behavior was monitored using a video dimension analysis method as described in "Materials and Methods." Panel A: Changes in beating behavior of a cell 10 minutes after exposure to buffer containing 5 nM phenylephrine and 5 nM propranolol. Panel B: Myocytes desensitized to AII by treatment with 100 nM AII for 10 minutes. As shown, the contractile behavior had returned to control value. At this point, the cells were challenged with 5 nM phenylephrine and 5 nM propranolol.

FIGURE 6. Angiotensin II (AII) desensitization of inositol phosphate accumulation after pretreatment with the G protein activator AlF₄⁻ or phenylephrine (Phe). Cells were pretreated with (hatched bars) or without (open bars) 100 nM AII for 10 minutes. After the cells were washed, they were incubated with 10 mM AlF₄⁻ for 30 minutes, 100 μM phenylephrine for 30 minutes, or 100 nM AII for 30 seconds. The accumulation of total [3H]inositol phosphates was measured as described in "Materials and Methods." The results are mean±SEM from five separate experiments. Pilot experiments revealed that the rate of total inositol phosphate accumulation stimulated by these agents was linear during the incubation times used (data not shown).
cesses that are independent of protein kinase C. This hypothesis was tested in cells that were depleted of protein kinase C. Myocytes were treated overnight with TPA under conditions that we have previously shown lead to a loss in enzyme activity of >96%.21 As shown in Figure 9A, in such cultures short-term TPA treatment (100 nM, 10 minutes) did not desensitize the cells to subsequent Ang II application, whereas the phosphoinositide pathway in these cells was desensitized after an Ang II pretreatment (100 nM, 10 minutes) in the normal manner (compare with Figure 9B). Figure 9B shows expected results from control cultures (grown in parallel with the protein kinase C-depleted cells) in which pretreatment with either Ang II (100 nM) or TPA (100 nM) yielded cells in which the phosphoinositide pathway was desensitized to a subsequent 30-second Ang II application. Thus, Ang II–evoked desensitization occurred in cells that were protein kinase C deficient.

One factor that limited interpretations in these experiments was that Ang II receptor–effector coupling had been assessed in 30-second intervals. Significant alterations in the time course of Ang II–evoked inositol phosphate accumulation may have also explained the results of Figure 9. Accordingly, time-course studies from 30 seconds to 5 minutes were performed (Figure 10). As expected, there was no measurable Ang II–evoked [3H]inositol phosphate accumulation for 5 minutes after short-term treatment with phorbol. In protein kinase C–depleted cells, there is a slight enhancement in the initial rate of [3H]inositol phosphate accumulation, yet desensitization, with a time course comparable to that of control cells, is still observed. These results confirmed that hormone-induced desensitization still occurred in protein kinase C–depleted cells.

**Discussion**

The present study examines the biochemical basis of Ang II–induced biological desensitization in heart muscle and the role of the phosphoinositide signaling system in this process. Cultured cardiac cells were used

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**Figure 8.** Effect of pretreatment with 12-O-tetradecanoylphorbol 13-acetate (TPA) on inositol phosphate accumulation after stimulation with G protein activator AlF₄⁻, phenylephrine (Phe), and angiotensin II (Ang II). Cells from a single culture were incubated in parallel with (hatched bars) or without (open bars) 100 nM TPA for 10 minutes at 37°C. After this preincubation, groups of cells were stimulated with 100 nM Ang II for 30 seconds, 10 mM AlF₄⁻ for 30 minutes, or 100 μM phenylephrine for 30 minutes. After these incubations, the levels of [3H]inositol phosphates were measured as described in "Materials and Methods." These data represent mean±SEM from five separate experiments.

**Figure 9.** Effects of angiotensin II (Ang II) and phorbol ester on desensitization of protein kinase C (PKC)-depleted cells. Cells were depleted of protein kinase C by treatment with 1 μM 12-O-tetradecanoylphorbol 13-acetate (TPA) for 18 hours as previously described.21 Panel A: PKC-depleted cells pretreated with either TPA or Ang II for 10 minutes as indicated. After the pretreatments, the cells were incubated with (hatched bars) or without (open bars) 100 nM Ang II for 30 seconds, and the resulting accumulation of [3H]inositol 1,4-bisphosphate was measured in extracts of the cells. Panel B: Results from experiments performed on cells that were grown in parallel and treated in an identical manner except that PKC was not depleted. The results are the means of duplicates±range/2. The experiments were repeated two times with identical results.
cardiocytes (M.M. Abdellatif and T.B. Rogers, unpublished observations). Thus, it is possible that functional responses require a high level of phosphoinositide turnover or that the other mechanisms of desensitization may be involved. These results also suggest that Ang II has long-term effects on cardiac function. Consistent with this interpretation, Baker et al.22 have reported that Ang II can stimulate hypertrophic responses in cardiac cells. It will be important to determine if such sustained phosphoinositide signaling underlies a growth factor function of Ang II in heart.

The combined biochemical/functional approach was useful in clearly defining a selective action for Ang II. Both functional and biochemical responses to the α-agonist phenylephrine were normal in Ang II-desensitized cells. A comparable agonist-specific desensitization of the β-adrenergic receptor/adenylate cyclase system has been well documented and has been termed "homologous."23 In the β-adrenergic system, receptor sequestration is not the underlying molecular mechanism of rapid, homologous desensitization.2 In the present study, the evidence derived from surface receptor measurements and blockers of receptor sequestration suggest that Ang II receptor transport from the cell surface is not a likely mechanism as well. Although Ang II receptor internalization may be important in some systems, such as vascular smooth muscle,9 the rapid, homologous desensitization of Ang II in heart appears similar to that reported for the β-adrenergic system.2 However, since the focus in the present studies was to examine rapid changes, it is possible that long-term receptor downregulation or sequestration may occur in heart cells.

Which Step in the Phosphoinositide Path Is Altered During Desensitization?

Signal transduction systems can desensitize if any critical step in the pathway is unresponsive or blocked. Thus, a major question arises in this study: Is the Ang II-evoked homologous desensitization a result of specific changes in receptor function, as seen in the β-adrenergic system, or a result of other crucial changes downstream in the pathway? Accordingly, the major components of the Ang II pathway, receptors, G proteins, phospholipase C, and protein kinase C, were examined in a systematic manner in a series of experiments. Although it is not possible to completely exclude the possibility that there are minor, Ang II-sensitive compartments of G proteins, phospholipase C, or PtdIns(4,5)P₂, it is most reasonable to conclude from these studies that the Ang II receptor is the locus for the rapid, homologous desensitization.

Role of Protein Kinase C in Desensitization

It is evident that activation of cardiac protein kinase C can lead to an uncoupling of hormone receptors from the phosphoinositide pathway. This might be expected, since it has been well established in many systems that pharmacological activation of protein kinase C with phorbol esters results in the
inhibition of agonist-stimulated PtdIns(4,5)P₂ hydrolysis.\textsuperscript{11,19,20} In the present study, short-term phorbol ester treatment resulted in a complex pattern of desensitization of the phosphoinositide pathway, with cells unresponsive to phenylephrine as well as to Ang II. Such a complex pattern in which cells are desensitized to multiple agonists has been previously defined as “heterologous” in the β-adrenergic/adrenalate cyclase system.\textsuperscript{21} It is likely that the effects of phorbol are mediated by protein kinase C, since TPA treatment failed to desensitize protein kinase C–depleted cells. The target for phorbol action in the pathway is likely to be upstream from G proteins or phospholipase C, perhaps at the level of the receptors, since AlF₆⁻–stimulated response was not attenuated in these cells. The fact that the α₁-adrenergic receptor has been reported to be a substrate for protein kinase C is consistent with this model.\textsuperscript{24} Thus, there are similarities between the Ang II system in heart and the β-adrenergic pathway in other tissues. In the latter system, activation of protein kinase A leads to heterologous desensitization, whereas receptor activation by agonist leads to a homologous pattern of desensitization.\textsuperscript{2,25}

A likely hypothesis might be that Ang II–evoked desensitization is mediated by protein kinase C. However, more detailed studies reported here indicate that this enzyme is not required for this process. The homologous pattern of hormone-evoked desensitization is very different from the heterologous one observed for phorbol ester. Further, protein kinase C–depleted cells are desensitized to Ang II in a manner analogous to that seen for normal cultures. In all, these results support the view that, although exogenous agents that activate protein kinase C can desensitize the phosphoinositide pathway, this signaling process is not promoted by agonist occupancy of the Ang II receptor. It is not possible to completely exclude the possibility that there is a small, specific pool of protein kinase C that is insensitive to phorbols and that can be recruited by Ang II stimulation. The results with the α₁-adrenergic agonists, which are very efficient activators of PtdIns(4,5)P₂ hydrolysis, and presumably stimulate protein kinase C, make this interpretation unlikely. These agonists fail to desensitize cardiocytes at either the biochemical or functional level.

In summary, application of Ang II to heart cells leads to a rapid homologous functional/biochemical desensitization. Although receptor sequestration is not an important mechanism, the locus of the desensitization is likely to be the Ang II receptor. The characteristics of this desensitization are similar to those seen with the β-adrenergic pathway. Since the importance of receptor phosphorylation is well documented in that system,\textsuperscript{25} it will be of interest to directly assess the importance of phosphorylation of the Ang II receptor in this process.

References


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