A βγ Dimer Derived from G_{13} Transduces the Angiotensin AT_{1} Receptor Signal to Stimulation of Ca^{2+} Channels in Rat Portal Vein Myocytes

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A G protein composed of \( \alpha_{13}, \beta_{1}, \) and \( \gamma_{3} \) subunits selectively couples the angiotensin AT_{1} receptors to increase cytoplasmic Ca^{2+} concentration ([Ca^{2+}]_{i}) in rat portal vein myocytes (Macrez-Leprêtre, N., Kalkbrenner, F., Morel, J. L., Schultz, G., and Mironneau, J. (1997) J. Biol. Chem. 272, 10095–10102). We show here that Gβγ transduces the signal leading to stimulation of L-type Ca^{2+} channels. Intracellular dialysis through the patch pipette of a carboxyl-terminal anti-β_{comb} antibody and a peptide corresponding to the Gβγ binding region of the β-adrenergic receptor kinase 1 inhibited the stimulation of Ca^{2+} channels and the increase in [Ca^{2+}]_{i} evoked by angiotensin II. The Gβγ binding peptide did not prevent the dissociation of the heterotrimeric G protein into its subunits, as it did not block activation of phospholipase C-β by Go_{α}, in response to stimulation of α_{1}-adrenoreceptors. Transient overexpression of the β-adrenergic receptor kinase 1 fragment and of Go_{α} subunits also inhibited the angiotensin II-induced increase in [Ca^{2+}]_{i}. Both anti-α_{13} antibody and carboxyl-terminal α_{13} peptide abrogated the angiotensin II-induced stimulation of Ca^{2+} channels. We conclude that activation of angiotensin AT_{1} receptors requires all three \( \alpha, \beta, \) and \( \gamma \) subunits of G_{13} for receptor-G protein interaction, whereas the transduction of the signal to L-type Ca^{2+} channels is mediated by Gβγ.

MEMBRANE CURRENT AND [Ca^{2+}]_{i}, MEASUREMENTS—Voltage clamp and membrane current recordings were made with a standard patch-clamp technique using a List EPC-7 patch-clamp amplifier (Darmstadt-Eberstadt, Germany). Whole-cell recordings were performed with patch pipettes having resistances of 1–3 megohms. Membrane potential and current records were stored and analyzed using an IBM PC computer (P-clamp system, Axon Instruments, Inc., Foster City, CA). Simultaneous recordings were obtained using an integrator (PCLAMP) and an A-D convertor (Blizard, Germany). Series resistance compensation was used and was monitored during recording. All experiments were performed with cells maintained in short-term primary culture in M199 medium containing 2% fetal calf serum, 2 mM glutamine, 1 mM pyruvate, 200 units/ml penicillin, and 200 μg/ml streptomycin; they were kept in an incubator gassed with 95% air, 5% CO₂ at 37 °C and used within 72 h.

Specific heterotrimeric G proteins composed of different \( \alpha, \beta, \) and \( \gamma \) subunits transmit signals from membrane receptors to intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2). When a receptor is activated by an agonist, it catalyzes the exchange of GDP for GTP on the intracellular effectors (1–2).

Membrane current and [Ca^{2+}]_{i}, Measurements—Voltage clamp and membrane current recordings were made with a standard patch-clamp technique using a List EPC-7 patch-clamp amplifier (Darmstadt-Eberstadt, Germany). Whole-cell recordings were performed with patch pipettes having resistances of 1–3 megohms. Membrane potential and current records were stored and analyzed using an IBM PC computer (P-clamp system, Axon Instruments, Inc., Foster City, CA). Simultaneous recordings were obtained using an integrator (PCLAMP) and an A-D convertor (Blizard, Germany). Series resistance compensation was used and was monitored during recording. All experiments were performed with cells maintained in short-term primary culture in M199 medium containing 2% fetal calf serum, 2 mM glutamine, 1 mM pyruvate, 200 units/ml penicillin, and 200 μg/ml streptomycin; they were kept in an incubator gassed with 95% air, 5% CO₂ at 37 °C and used within 72 h.

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1 The abbreviations used are: βARK, β-adrenergic receptor kinase; [Ca^{2+}]_{i}, cytoplasmic Ca^{2+} concentration; AT, angiotensin II; GFP, green fluorescent protein; CGP 42112, N-α-nicotinoyl-Tyr-Lys[N-α-CBZ-Arg]-His-Pro-Ile-OCOH; InsP₃, inositol triphosphate; WT, wild type.
ous measurements of intracellular Ca$^{2+}$ concentration were carried out in some experiments. Briefly, 60 μM fura-2 was added to the pipette solution and entered cells after establishment of the whole-cell recording mode. [Ca$^{2+}$], was estimated from the 340/380-nm fluorescence ratio using a calibration determined within cells (15). All measurements were made at 25 ± 1 °C.

Measurements of Cytosolic Ca$^{2+}$—Cells were loaded by incubation in physiological solution containing 1 μM fura-2-acectoxyethyl ester for 30 min at room temperature. These cells were washed and allowed to cleave the dye to the active fura-2 compound for at least 1 h. Fura-2 loading was usually uniform over the cytoplasm, and compartmentalization of the dye was never observed. Measurement of cytosolic Ca$^{2+}$ concentration was carried out by dual-wavelength fluorescence method, as described previously (15). Briefly, fura-2-loaded cells were mounted in a perfusion chamber and placed on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan). Single cells were alternatively excited with UV light at 340 and 380 nm through a 10-μm long-pass filter with a charge-coupled device camera (Hamamatsu Photonics, Hamamatsu City, Japan). The signal was processed (Hamamatsu DVS 3000) by correcting each fluorescence image for background fluorescence and calculating 340/380-nm fluorescence ratios on a pixel-to-pixel basis. Averaged frames were usually collected at each wavelength every 0.5 s. [Ca$^{2+}$], was calculated from mean ratios using a calibration determined in loaded cells. All measurements were made at 25 ± 1 °C.

Transfection—cDNA encoding β-adrenergic receptor kinase was cloned into expression plasmids pRK5 (8). cDNA encoding for Go$\alpha$, and Go$\alpha_{12}$ subunits were cloned into pECE (16). cDNA encoding for S65T green fluorescent protein was cloned into pcDNA3 (CLONTECH, Palo Alto, CA). Briefly, plasmids were diluted with water from stock solutions (0.5 μg/μl) to final concentrations of 0.1 μg/μl and injected directly into the nucleus of vascular myocytes. The S65T green fluorescent protein (GFP) was included to facilitate later identification of myocytes having a successful nuclear injection and plasmid expression. Fluorescence produced by the S65T GFP was observed 3 days after injection with a confocal microscope (Bio-Rad MRC 1000, Paris, France). From injected cells (n = 280), about 20% showed a detectable fluorescence signal.

Solutions—The normal physiological solution contained 130 mM NaCl, 5.6 mM KCl, 1 mM MgCl$\text{II}$, 2 mM CaCl$\text{II}$, 11 mM glucose, 10 mM HEPES, pH 7.4, with NaOH. The basic pipette solution contained 130 mM CsCl, 10 mM HEPES, pH 7.4, with CsOH. Ca$^{2+}$-free external solution was prepared by omitting CaCl$\text{II}$ and adding 0.5 mM EGTA. For the recording of Ca$^{2+}$ channel current, 5 mM BaCl$\text{II}$ was substituted for CaCl$\text{II}$ in the reference solution, and CaCl$\text{II}$ was used in the pipette and external solutions to block outward potassium currents. In addition, 10 mM EGTA, 5 mM Na$_2$ATP, and 1 mM MgCl$\text{II}$ were added to the basic pipette solution. Substances were applied to the cells by pressure ejection from a glass pipette for the period indicated on the records.

Chemicals and Drugs—M199 medium was from Flow Laboratories (Paisley, UK). Fura-2/AM, carboxyl-terminal Go$\alpha_{12}$ peptide (LHDNLKQLMLQ) and anti-α$_1$-antiserum were from Calbiochem (Meudon, France). Norepinephrine, rauwolscine, and propranolol were from Sigma. Angiotensin II and CGP 42112A (N-nicotinoyl-Tyr-Lys[ε- CBZ-Arg]-His-Pro-Ile-OH) was from Neosystem Laboratories (Strasbourg, France). Streptomyacin, penicillin, glutamate, and glutamate were from Life Technologies, Inc. (Paisley, UK). Fura-2/AM, carboxyl-terminal Go$\alpha_{12}$ peptide (LHDNLKQLMLQ) and anti-α$_1$-antibody were from Calbiochem (Meudon, France). Norepinephrine, rauwolscine, and propranolol were from Sigma. Angiotensin II and CGP 42112A (N-nicotinoyl-Tyr-Lys[ε- CBZ-Arg]-His-Pro-Ile-OH) was from Neosystem Laboratories (Strasbourg, France). Anti-α$_1$, and anti-α$_1$-antiserum were given by B. Nürnberg (University of Berlin). Anti-β$_{2,3}$ (raised to the carboxyl-terminal amino acids, TDDGMDAVGTSWSDKFLKQWN, of Go$\beta_\text{II}$ subunit) was from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-α$_{11}$ antibody was a gift from G. Guillou (INSERM U401 Montpellier, France). Carboxyl-terminal Go$\alpha_{12}$ peptide (LQLNLKEYNLV) and peptides corresponding to the Go$\beta_\text{II}$ binding domain of JARK (peptide G) were generated by Genosys (Cambridge, UK).

Data Analysis—Results are expressed as means ± S.E. Significance was tested by means of Student’s t test. P values of <0.05 were considered as significant.

RESULTS

Identification of the G Protein-coupling Angiotensin AT$_1$ Receptors to Stimulation of L-type Ca$^{2+}$ Channels—To identify the G proteins activated by the angiotensin AT$_1$ receptor, we used antibodies raised against the carboxyl-terminus of α-subunit to block interactions of G proteins with angiotensin AT$_1$ receptors and synthetic peptides corresponding to the carboxyl terminus of α-subunit to disrupt the angiotensin AT$_1$, receptor-evoked activation of G proteins. In the continuous presence of 100 nM CGP 42112A (to block angiotensin AT$_1$ receptors; Ref. 17), 10 nM AII increased the Ba$^{2+}$ current by about 40% (Fig. 1A). The stimulatory effect of AII reached a steady state within 1–2 min and was progressively reversed within 5–10 min. When anti-α$_1$-antiserum (at 1:100 or 1:50) was added to the pipette solution for 7–10 min, the AII-induced stimulation of the Ba$^{2+}$ current was not significantly affected (Fig. 1B). Similarly, intracellular applications of anti-α$_1$-antiserum (1:100) or anti-α$_1$-purified antibody (10 μg/ml) blocked the AII-induced stimulation of the Ba$^{2+}$ current. Fig. 2 illustrates the effects of synthetic peptides corresponding to the carboxyl-terminus of α-subunit.
FIG. 2. Effects of carboxyl-terminal G<sub>Q11</sub> and G<sub>Q12</sub> peptides on the AII-induced stimulation of Ca<sup>2+</sup> channel current. Ca<sup>2+</sup> channel current (measured with the whole-cell patch clamp) was evoked by a depolarization to 0 mV from a holding potential of −60 mV. A, Ca<sup>2+</sup> channel current under control conditions (1) and after dialysis of 0.1 μg/ml α<sub>Q11</sub> peptide (2) or 1 μg/ml α<sub>Q11</sub> peptide (3) for 7–8 min before (a) and during application of 10 nM AII (b). B, compiled data showing the effects of increasing concentrations of α<sub>Q11</sub> peptide and of α<sub>Q11</sub> peptide on AII-induced stimulation of Ca<sup>2+</sup> channel currents, expressed as a fraction of the current amplitude before stimulation (I/I<sub>c</sub>). Results are means ± S.E. obtained from two different cell batches. In parentheses: number of cells tested. [star], values significantly different from control values (p < 0.05). External solution contained 5 mM Ba<sup>2+</sup> and 100 mM CPG 42112A.

FIG. 3. Effects of anti-β<sub>com</sub> antibody and βARK peptides on the AII-induced stimulation of Ca<sup>2+</sup> channel current. Ca<sup>2+</sup> channel current (measured with the whole-cell patch clamp) was evoked by a depolarization to 0 mV from a holding potential of −60 mV. A, Ca<sup>2+</sup> channel current under control conditions (1) and after dialysis of 10 μg/ml anti-β<sub>com</sub> antibody (2) or boiled (95 °C for 30 min) anti-β<sub>com</sub> antibody (3) into the cells for 7–8 min before (a) and during application of 10 nM AII (b). B, compiled data showing the effects of increasing concentrations of peptide G (corresponding to the Gβγ binding domain of βARK<sub>1</sub>) and of 10 μg/ml peptide A (corresponding to a domain of βARK<sub>1</sub> not involved in Gβγ binding) on the AII-induced stimulation of Ca<sup>2+</sup> channel currents, expressed as a fraction of the current amplitude before stimulation (I/I<sub>c</sub>). Results are means ± S.E. obtained from three different cell batches. In parentheses: number of cells tested. [star], values significantly different from control values (p < 0.05). External solution contained 5 mM Ba<sup>2+</sup> and 100 mM CPG 42112A.

peptides corresponding to the carboxyl terminus of α<sub>Q11</sub> and α<sub>Q13</sub> subunits. Intracellular applications of the α<sub>Q13</sub> peptide for 7–8 min inhibited the AII-induced stimulation of the Ba<sup>2+</sup> current in a concentration-dependent manner (Fig. 2, A and B). The concentration of α<sub>Q13</sub> peptide producing half-maximal inhibition was estimated to be 4 ng/ml. Complete inhibition was obtained with 0.1 μg/ml α<sub>Q13</sub> peptide. It has to be noted that the α<sub>Q13</sub> peptide by itself had no effect on the Ba<sup>2+</sup> current, as the current density, normalized by the cell capacitance, was not significantly modified (control: 12.5 ± 2.5 microampere/microfarad, n = 10; in the presence of 1 μg/ml α<sub>Q13</sub> peptide: 13.2 ± 3.5 microampere/microfarad, n = 8). Intracellular applications of α<sub>Q11</sub> peptide (0.1–1 μg/ml) had no effect on the AII-induced stimulation of the Ba<sup>2+</sup> current (control: 46 ± 5%, n = 10; in the presence of 1 μg/ml α<sub>Q11</sub> peptide: 44 ± 4%, n = 6). Taken together, these results suggest that G<sub>Q13</sub>, but not G<sub>Q12</sub> and G<sub>Q11</sub>, functionally couples the angiotensin AT<sub>1</sub> receptors to stimulation of L-type Ca<sup>2+</sup> Channels.

Gβγ Is Required for AII-induced Stimulation of Ca<sup>2+</sup> Channels—Anti-Gα antibody and Gα subunit peptide block of the response alone cannot distinguish which G protein subunit (Gα or Gβγ) transduces the signal to Ca<sup>2+</sup> channels. To determine which G protein subunit was involved in effector activation, an anti-β<sub>com</sub> antibody (18), raised to the carboxyl terminus of GB<sub>3</sub> subunit, was dialyzed into the cell for 7–8 min. As shown in Fig. 3A, intracellular applications of 10 μg/ml anti-β<sub>com</sub> antibody blocked the AII-induced stimulation of the Ba<sup>2+</sup> current. In contrast, application of the same concentration of boiled anti-β<sub>com</sub> antibody (95 °C for 30 min) had no significant effect on the AII-induced stimulation of the Ba<sup>2+</sup> current (Fig. 3A).

In a second set of experiments, we dialyzed peptides corresponding to fragments of βARK<sub>1</sub> (19) into the cells for 5–6 min. Carboxyl-terminal fragments of βARK<sub>1</sub> have been used to bind Gβγ subunits and to block activation of effectors (19–21). Intracellular applications of peptide G (corresponding to the Gβγ binding domain of βARK<sub>1</sub>) inhibited the AII-induced stimulation of the Ba<sup>2+</sup> current in a concentration-dependent manner (Fig. 3B). The concentration of peptide G producing half-maximal inhibition was estimated to be 65 nM. Complete inhibition was obtained with 1 μM peptide G.
Inhibition of the AII-dependent Ca\(^{2+}\) signaling by binding of Gβγ could be related to two distinct mechanisms. (i) The inhibitory proteins (βARK-K peptide, anti-β-com antibody) prevented the AII-induced dissociation of Gα\(_{12}\) and Gβγ subunits, thus inhibiting the transduction process or (ii) they bind to Gβγ after its release from Go and, therefore, inhibit the Gβγ-effector coupling. To distinguish between these possibilities, the cells were stimulated by 10 μM norepinephrine to produce an increase in \([\text{Ca}^{2+}]_m\), dependent on Ca\(^{2+}\) release from the intracellular store. This transduction pathway involves Gα\(_q\), which activates a phospholipase C-β, leading to InP\(_{3}\) production and the subsequent activation of InP\(_{3}\)-gated channels (15). As illustrated in Fig. 4B, intracellular applications of 10–100 μM peptide G to scavenge free Gβγ had no effect on the norepinephrine-induced Ca\(^{2+}\) release that was mediated by the α\(_q\) subunit. Moreover, intracellular applications of both peptide G (100 μM) and α\(_{11}\) Peptide (10 μg/ml) inhibited the norepinephrine-induced Ca\(^{2+}\) release (Fig. 4B). These results show that, although Gβγ is bound to the peptide G, Gα\(_q\) on its own can transduce and support the Ca\(^{2+}\) response evoked by norepinephrine.

In a second set of experiments, we overexpressed a carboxyl-terminal fragment of βARK-I by intranuclear microinjection of expression plasmids containing cDNA inserts coding for βARK-I fragment and S65T GFP. Overexpression of the βARK-I fragment was followed by cytoplasmic detection of S65T GFP, 3 days after injection. As illustrated in Fig. 5A, the AII-induced increase in \([\text{Ca}^{2+}]_m\), was inhibited by about 75% in cells overexpressing the βARK-I fragment (control: 103 ± 13 nM, n = 20; after βARK-I fragment overexpression: 25 ± 9 nM, n = 12). It has to be noted that in all the cells injected with expression plasmids, the basal \([\text{Ca}^{2+}]_m\) level was significantly increased (control: 47 ± 5 nM, n = 50; after plasmid injection: 88 ± 5 nM, n = 56). However, in cells injected with pRK5 plasmids alone, AII evoked a Ca\(^{2+}\) response whose amplitude (98 ± 9 nM, n = 12) was not significantly different from that measured in non-injected cells (Fig. 5B).

Another strategy to confirm the role of Gβγ in AII-induced increase in \([\text{Ca}^{2+}]_m\), was to increase the normal Gα-Gβγ ratio by overexpressing Gα subunits. Excess of GDP-bound Gα subunits with a high affinity for Gβγ subunits should create conditions favoring heterotrimeric formation. Injection of expression plasmids containing cDNA inserts coding for wild type (WT) Gα\(_{11}\) (which is not endogenously expressed in vascular myocytes; Ref. 22) or WT Gα\(_{12}\) inhibited the AII-induced increase in \([\text{Ca}^{2+}]_m\), by about 75% (Fig. 5B). In contrast, overexpression of the constitutively active Q229L Gα\(_{12}\) did not significantly affect the AII-induced increase in \([\text{Ca}^{2+}]_m\). Taken together, these results indicate that the idea that Gβγ controls the transduction pathway, leading to stimulation of Ca\(^{2+}\) channels and increase in \([\text{Ca}^{2+}]_m\), in response to activation of angiotensin AT\(_1\) receptors.

**DISCUSSION**

Our results show that, in rat portal vein myocytes, G\(_{13}\) couples angiotensin AT\(_1\) receptors to stimulation of L-type Ca\(^{2+}\) channels. The Gα\(_{12}\)β\(_{13}\) heterotrimer provides the specificity for the coupling with AT\(_1\) receptors (11), but the signal to Ca\(^{2+}\) channel is transduced by the Gβγ dimer. This conclusion is based on experiments using antibodies raised against the carboxy-terminal Gα or Gβ subunits, synthetic peptides corresponding to the carboxy termini of Gα subunits or to βARK-K fragments, and overexpression of a βARK-K fragment and of Gα subunits to disrupt the angiotensin AT\(_1\) receptor-evoked activation of Ca\(^{2+}\) channels.

Involvement of Gα\(_{11}\) in the Ca\(^{2+}\) responses evoked by AII is demonstrated by intracellular perfusion of an anti-α\(_{11}\) antibody.
that selectively blocks the AII-induced stimulation of Ca\(^{2+}\) channels and the increases in [Ca\(^{2+}\)]. These results support the idea that activation of Ca\(^{2+}\) channels via G\(_{\alpha 13}\) is the initial step leading to an increase in [Ca\(^{2+}\)]. To confirm these results, we used synthetic peptides corresponding to the carboxyl terminus of G\(_{\alpha}\) subunits. These peptides have been shown to bind to receptors and to stabilize them in a high affinity conformational state (23–25). Thus, synthetic peptides may act as competitive agonists at the receptor/G protein interface and block receptor-mediated activation of effectors (26). As carboxyl-terminal G\(_{\alpha 13}\) peptide selectively abrogates the AII-induced stimulation of Ca\(^{2+}\) channels whereas carboxyl-terminal G\(_{\alpha 11}\) peptide is ineffective, our results suggest that the activated angiotensin AT1 receptors specifically interact with the extreme carboxyl terminus of G\(_{\alpha 13}\) to promote dissociation of the heterotrimer involved in the regulation of [Ca\(^{2+}\)].

Involvement of G\(_{\beta\gamma}\) in Ca\(^{2+}\) channel stimulation is supported by the following results. (i) The anti-\(\beta_{1}\)om antibody inhibited the AII-induced stimulation of Ca\(^{2+}\) channels and increase in [Ca\(^{2+}\)]; (ii) the \(\beta\)ARK\(_1\) peptide (corresponding to the G\(_{\beta\gamma}\) binding domain of \(\beta\)ARK\(_1\)) specifically abrogated the AII-mediated responses; (iii) transient overexpression of a \(\beta\)ARK\(_1\) fragment and of G\(_{\alpha\gamma}\) and G\(_{\alpha 12}\) inhibited the AII-induced Ca\(^{2+}\) response. These experiments clearly show that G\(_{\beta\gamma}\) is necessary to mediate the AII-induced increase in [Ca\(^{2+}\)]. The fact that the inhibitory proteins interacting either with G\(_{\beta\gamma}\) or Go inhibit the AII-induced Ca\(^{2+}\) signaling may indicate that they bind to the undissociated heterotrimer and prevent the dissociation of the G protein into its subunits. Another possibility is that the inhibitory proteins bind to the dissociated G protein subunits and suppress their action on the effectors. This latter possibility is supported by our observations that overexpression of G\(_{\alpha}13\) and G\(_{\alpha 12}\) (which cannot bind to heterotrimeric G proteins) inhibited the AII-induced Ca\(^{2+}\) responses. In addition, intracellular applications of the \(\beta\)ARK\(_1\) peptide G did not inhibit the norepinephrine-activated Ca\(^{2+}\) response as would be expected if this peptide prevented the G protein heterotrimer dissociation. Although G\(_{\beta\gamma}\) is bound to the peptide G, G\(_{\alpha}13\) on its own can transduce and support Ca\(^{2+}\) release from the intracellular store in response to activation of \(\alpha 1\)-adrenoreceptors (27). Taken together, these observations indicate that these inhibitory proteins interact with the free G protein subunits and, thus, suppress their actions on the effectors.

Coupling of G proteins with Ca\(^{2+}\) channels has been previously reported and generally promotes an inhibition of neuronal Ca\(^{2+}\) currents (28–30). This inhibition can result from a direct interaction between G\(_{\beta\gamma}\) and the pore-forming \(\alpha 1\) subunits of N- and P/Q-type Ca\(^{2+}\) channels but not of L-type Ca\(^{2+}\) channels (31). In contrast, L-type Ca\(^{2+}\) currents in cardiac and vascular myocytes can be enhanced by phosphorylation by protein kinases A and C, probably on phosphorylation sites located on the carboxyl terminus of the \(\alpha 1\) subunit or on the \(\beta\) subunit of Ca\(^{2+}\) channels (32, 33). Protein kinase C has been reported to increase Ca\(^{2+}\) channel activity through the modulation of the mean open time, a voltage-independent property, whereas protein kinase A enhances channel activity through the modification of the voltage-dependence of activation and inactivation (34). In portal vein myocytes, AII increases the Ca\(^{2+}\) channel current at all potentials but does not shift the current-voltage relationship. However, the relative increase in Ca\(^{2+}\) current induced by AII is more pronounced at negative potentials, i.e., between −40 and −20 mV, than at 0 mV, supporting our previous data that the AII-induced stimulation of Ca\(^{2+}\) channels involves activation of protein kinase C (12).

G\(_{\beta\gamma}\)-induced Ca\(^{2+}\) signaling may also involve various effector systems leading to second messenger production. It has been reported in several cell types that G\(_{\beta\gamma}\)-mediated activation of phospholipase C-\(\beta_2\) or -\(\beta_3\) is associated with a relatively low increase in Inos\(_3\) (35). Moreover, G\(_{\beta\gamma}\) may increase the apparent affinity of the Inos\(_3\)-gated Ca\(^{2+}\) channels to Inos\(_3\), so that Ca\(^{2+}\) release may occur at low Inos\(_3\) concentrations (36). Recently, it has been shown that G\(_{\beta\gamma}\) is involved in the regulation of the activity of the small GTP-binding proteins Rho and Rac (37). Thus, G\(_{\beta\gamma}\) may mediate the association of activated Rho and Rac to the membrane and activate, in turn, various enzymes such as phospholipase D or phosphoinositide 3-kinase (38–40), leading to generation of various second messengers. The specificity of the G\(_{\beta\gamma}\)-activated coupling could depend on localized distribution of certain subsets of receptors, G proteins, and effectors within distinct membrane domains that may have access to each other (41). Our results suggest that G\(_{\beta\gamma}\) activates L-type Ca\(^{2+}\) channels by a transduction pathway that does not involve Inos\(_3\)-gated Ca\(^{2+}\) release channels, since the AII-induced increase in [Ca\(^{2+}\)] is insensitive to both heparin (an inhibitor of the Inos\(_3\) receptor) and anti-phosphatidylinositol antibody (19). In addition, biochemical and pharmaco-
logical approaches have proposed that AII may induce phosphatidylincholine hydrolysis by phospholipases D (42) or C (12, 43), leading to diacylglycerol formation. Release of diacylglycerol from phosphatidylincholine hydrolysis is a slow phenomenon (42), supporting the observation that the AII-induced stimulation of Ca2+ channel current reaches a maximum value within 1–2 min. Activation of protein kinase C by diacylglycerol may be responsible for phosphorylation of L-type Ca2+ channels and the subsequent increase in Ca2+ channel activity (34). In vascular myocytes, openings of L-type Ca2+ channels at the resting potential (~50 mV) have been previously proposed to serve as a pathway for Ca2+ influx in response to receptor activation (44, 45).

In conclusion, we show that in rat portal vein myocytes, activation of angiotensin AT1 receptors requires G13 heterotrimer for specificity and Gβγ dimer for transducing the signal to L-type Ca2+ channels and increase in [Ca2+]i.

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