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A G Protein Is Involved in the Angiotensin AT_2 Receptor Inhibition of the T-Type Calcium Current in Non-differentiated NG108–15 Cells*

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In non-differentiated NG108–15 cells, both angiotensin II (Ang II) (100 nM) and CGP 42112 (100 nM) decreased the T-type calcium current amplitude by 24 ± 2% and 21 ± 3%, respectively. cGMP is not a mediator of the Ang II effect, since loading of cells with 50 μM cGMP did not prevent the inhibitory effects of Ang II. The effects of Ang II involves a non-identified GTPase activity since incubation with GDPβS (3 mM) completely reversed the inhibitory effect of Ang II while GTPγS mimicked its effect. However, Ang II binding was not affected by GTPγS, and the effect of Ang II was not modified in pertussis toxin-treated cells. The inhibitory effect of Ang II on the T-type Ca^{2+} current involves a phosphotyrosine phosphatase activity since sodium orthovanadate prevented the effects of Ang II, although microcystin-LR, a selective Ser/Thr phosphatase 1 and 2A inhibitor, did not modify the effect of Ang II. These results provide the first evidence of a modulation of membrane conductance by Ang II through the AT_2 receptor and demonstrate the involvement of a phosphotyrosine phosphatase and a G protein in the AT_2 transduction mechanism in NG108–15 cells. Moreover, our data suggest that phosphotyrosine phosphatase activation is proximal to receptor occupation, since sodium orthovanadate inhibits both GTPase activity and T-type current blockage induced by Ang II or CGP 42112, while GTPγS inhibition of the T-type calcium current is not impaired.

Pharmacological studies have clearly identified two classes of angiotensin II (Ang II)[1] receptors. The AT_1 receptor is closely associated with cardiovascular regulation, fluid volume homeostasis, and cellular growth (1, 2). Activation of the AT_1 receptor is linked to phospholipase C activation and Ca^{2+} influx, effects which are mediated by G proteins (1, 2). The AT_2 receptors have been identified in many fetal tissues, including brain (3–8) and cell lines of neuronal origin (9–11). In contrast with the AT_1 receptor, no physiological function has yet been attributed to the AT_2 receptor, and its transduction signaling pathway(s) is a much debated question (1, 2). Stimulation of the AT_2 receptor does not stimulate inositol phosphate accumulation, release of Ca^{2+} from intracellular stores, cAMP production, or arachidonic release (10–12). Ang II binding on AT_2 receptors has been shown to decrease intracellular cGMP levels (13–15). Two mechanisms have been proposed as mediators of this decrease: a phosphodiesterase activation through calcium entry (16) or the inhibition of a particulate guanylate cyclase through phosphotyrosine phosphatase (PTPase) activity (1, 14, 17). However, this AT_2-mediated cGMP decrease is not observed in all studies (10, 11, 18). Results regarding AT_2 modification of cellular phosphotyrosine patterns are even more controversial; some authors observe no change in tyrosine phosphorylation (10) while others describe an increased phosphorylation of tyrosine residues (19, 20), and Bottari *et al.* (14) and Brechler *et al.* (17) report the stimulation of a PTPase activity. The angiotensin AT_2 receptor does not interact with guanine nucleotide binding proteins (G proteins) in PC12W cells (21), in ovarian granulosa cells (18), nor in fetal skin or fetal skeletal muscle (22). Nevertheless, binding experiments using rat brain suggest that two subclasses of AT_2 receptors can be distinguished: AT_{2A} for receptors that are sensitive to guanine nucleotides and pertussis toxin (PTX) and AT_{2B} for receptors that are not (6). Substantial differences in binding of [^{125}I]CGP 42112 between brain and adrenal AT_2 receptors have also been reported (23). Recently, the AT_2 receptor was cloned by two different groups (24, 25). Both found that the AT_2 receptor belongs to the seven-transmembrane domain receptor class with a low homology (32–34%) with the AT_1 receptor.

We previously reported the Ang II modulation of the T-type calcium current in non-differentiated NG108–15 cells expressing only the angiotensin AT_2 receptor type (26). In the present study, we show that, in this cell line, the inhibitory effect of Ang II on the T-type Ca^{2+} current by Ang II is not dependent on intracellular cGMP concentration, is not PTX-sensitive, but is abolished by GDPβS and involves a phosphotyrosine phosphatase activity. Moreover, to correlate the effects of Ang II on T-type current with signaling pathways, we demonstrate in the same experimental conditions that Ang II decreases the level of phosphotyrosine proteins and stimulates a membraneous GTPase activity. It could be concluded that the signaling pathway of the AT_2 receptor involves a PTPase and yet unknown G protein, which regulates the T-type current.

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[1] The abbreviations used are: Ang II, angiotensin II; PTX, pertussis toxin; PTPase, phosphotyrosine phosphatase; DADLE, [D-Ala^2,D-Leu^5]enkephalin; GDPβS, guanosine 5′-*O*-2-(thio)diphosphate; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate.

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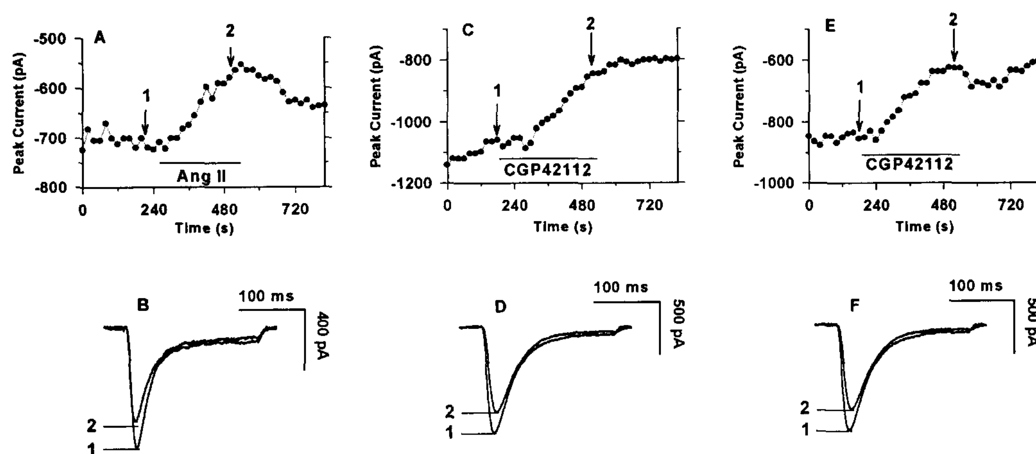

FIG. 1. **Effect of Ang II and of the AT_2 selective ligand CGP 42112 on the T-type calcium current.** *A* and *B*, effect of Ang II in control medium. *A*, plot of the peak current *versus* time; Ang II (100 nM) was applied as indicated by the *dark line*. *B*, currents recorded before (*1*) and after (*2*) Ang II addition. *C* and *D*, effect of CGP 42112 in control medium. *C*, plot of the peak current *versus* time; CGP 42112 (100 nM) was applied as indicated by the *dark line*. *D*, currents recorded before (*1*) and after (*2*) CGP 42112 addition. *E* and *F*, effect of 50 μM cGMP added into the pipette solution. *E*, plot of the peak current *versus* time; CGP 42112 (100 nM) was applied as indicated by the *dark line*. *F*, currents recorded before (*1*) and after (*2*) CGP 42112 addition. Intracellular buffering of the cGMP concentration did not affect the T-type Ca^{2+} current modulation by CGP 42112. 200 ms depolarizing steps were delivered every 20 s from −80 to −25 mV.

MATERIAL AND METHODS

Solutions and Chemicals

Tetrodotoxin, 8-bromo-cGMP, and sodium orthovanadate were purchased from Sigma. ATP, GTP, GTPγS, and GDPβS were purchased from Boehringer-Mannheim, and microcystin-LR was purchased from Life Technologies, Inc. Pertussis toxin was from List Biological Laboratories, [Val^5]-angiotensin II and [D-Ala^2,D-Leu^5]enkephalin were from Peninsula Peptides, anti-phosphotyrosine antibody (monoclonal antibody, IgG2bk) was from UBI (Lake Placid, NY), and CGP 42112 was provided by Ciba-Geigy (Basel, Switzerland). Sodium pervanadate was prepared just before the experiment by mixing vanadate and H_2O_2 (5:1). The cells were preincubated 5 min in the pervanadate-supplemented medium and then washed with fresh extracellular solution prior to the patch clamp studies.

Cell Culture

NG108–15 cells (provided by Drs. M. Emerit and M. Hamon; INSERM, U. 238, Paris) were cultured in Dulbecco's modified Eagle's medium as previously described (26). For patch clamp recordings, cells were used between 24 and 48 h after subculture, and the cell density was 1×10^4 cells/35-mm Petri dish. For biochemical studies, cells were used under the same conditions but at a higher density (2×10^6 cells/Petri dish).

Whole Cell Voltage Clamp

The extracellular solution consisted of (in mM) 100 NaCl, 10 $CaCl_2$, 1 $MgCl_2$, 5 CsCl, 35 tetraethylammonium chloride, 5 HEPES, and 0.5 μM tetrodotoxin, pH 7.3, with NaOH. The intracellular solution consisted of (in mM) 20 NaCl, 120 CsCl, 1 $CaCl_2$, 11 EGTA, 2 $MgCl_2$, 5 HEPES, 3 ATP, and 0.2 GTP, pH 7.3, with CsOH. The osmolarity was adjusted to 300 mosM. All experiments were conducted at room temperature (22–24 °C), as previously described (26). Ionic current recordings were obtained using the whole cell configuration of the patch clamp method with an Axopatch 1B amplifier (Axon Instrument, Foster City, CA) piloted by pClamp software (Axon Instrument) on an IBM-PC computer. The junction potential, as well as the capacitive transient, was compensated for. Cell series resistance and capacity were empirically compensated. Currents were low-pass filtered at 1 kHz and sampled at 4 kHz.

Biochemical Studies

Membrane Preparation for Ang II Binding and ADP-ribosylation—Membrane preparation, binding studies, and ADP-ribosylation experiments have been conducted as previously described (14, 15, 26, 27).

Analysis of Tyrosine-phosphorylated Proteins—Experiments were performed as previously described (14). After washing procedure, cells (2×10^6 in 3.5-cm Petri dishes) were incubated with or without stimuli and were lysed; proteins were then processed for Western blot. The phosphotyrosine-containing proteins were detected using the anti-phosphotyrosine antibody (diluted 1:650) together with horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence detection system (Amersham Corp.).

Analysis of GTPase Activity—GTPase assay was performed according to the method described by Cassel and Selinger (28) and consisted of measuring the release of ^{32}P from [γ-^{32}P]GTP.

RESULTS AND DISCUSSION

Angiotensin II and CGP 42112 Both Decrease the T-type Calcium Current

As shown in Fig. 1, Ang II and the AT_2 selective ligand CGP 42112 are both effective in inhibiting the T-type calcium current recorded at −25 mV (holding potential, −80 mV) where it is at maximal amplitude. Ang II (100 nM) decreases the T-type calcium current by 24 ± 2% (n = 23) (Fig. 1, *A* and *B*) and CGP 42112 (100 nM) by 21 ± 3% (n = 10) (Fig. 1, *C* and *D*). As previously shown (26), the reversibility was good although very slow. It could also be noted that the time course of Ang II inhibition is slow requiring 2–4 min to reach steady-state levels. Similar time courses were obtained for the blocking effect of Ang II (through the AT_1 receptor) on Ca^{2+} current from rat neurons (29). The CGP 42112-mediated inhibition of the T-type calcium current demonstrates the AT_2 specificity of the Ang II effect. Because Ang II and CGP 42112 show equal potencies in decreasing the T-type calcium current, both peptides were used in our investigations.

cGMP Does Not Affect AT_2 Modulation of the T-type Calcium Current

The decrease in intracellular cGMP is the less controversial of all the AT_2-mediated effects (1, 2) and has been described in neuronal cells (13, 16) and in the neuronal PC12W cell line (14, 17). We therefore first investigated the possible role of cGMP in the T-type calcium current modulation through AT_2 receptor stimulation. The intracellular concentration of cGMP is equal to or less than 0.1 μM in resting cells (30). To ascertain that the cGMP concentration will not decrease below this level under AT_2 receptor stimulation, we performed experiments where intracellular cGMP concentration was maintained at 50 μM by adding the non-hydrolyzable cGMP analog 8-bromo-cGMP to the pipette medium. As shown in Fig. 1, *E* and *F*, a high intracellular cGMP concentration does not affect T-type calcium current inhibition by CGP 42112 (20 ± 3% of decrease, n = 7). Thus, T-type current inhibition does not result from a decrease in intracellular cGMP concentration. It is therefore very unlikely that cGMP is involved in the AT_2 modulation of

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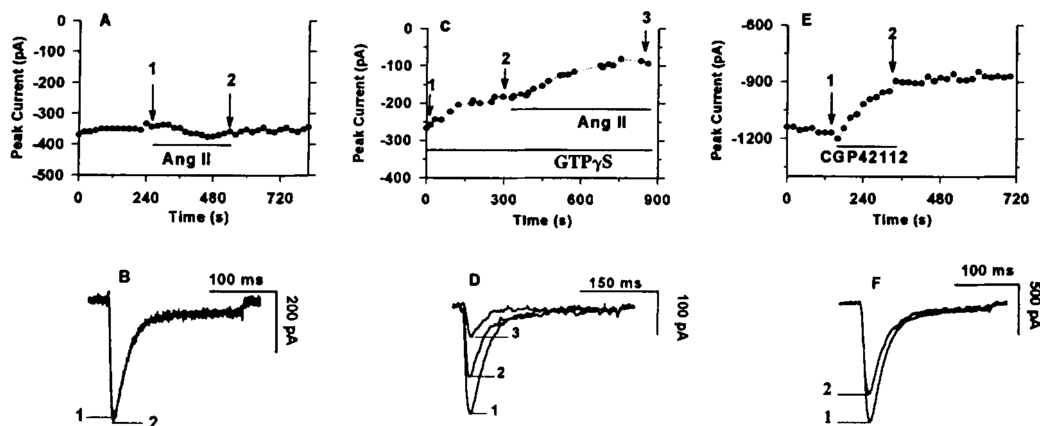

FIG. 2. **Effects of GDPβS, GTPγS, and PTX on the effect of Ang II on the T-type calcium current.** *A*, replacement of GTP by GDPβS (3 mM) into the standard intracellular solution; plot of the peak current *versus* time is shown. *B*, currents before (*1*) and after (*2*) the addition of Ang II (100 nM) as indicated in *panel A*. *C*, replacement of GTP by GTPγS (3 mM) into the standard intracellular solution. The current amplitude decreased slowly as soon as the patch was established and reached a steady state after 3 min. At the plateau level, addition of Ang II (100 nM) induced a further decrease of the current amplitude. *D*, control current trace (*1*) at the plateau level in presence of GTPγS (*2*) and after Ang II addition (3). *E* and *F*, effect of Pertussis toxin treatment on CGP 42112 modulation of the T-type calcium current. *E*, plot of the peak current *versus* time; *F*, currents before (*1*) and after (*2*) the addition of CGP 42112 (100 nM) as indicated for *panel C*; 200 ms depolarizations from −80 to −25 mV were delivered every 20 s.

the T-type calcium current in non-differentiated NG108–15 cells.

A G Protein Mediates the AT$_2$ Inhibition of the T-type Calcium Current

Effects of Guanine Nucleotides GDPβS and GTPγS on Ca^{2+} Current Inhibition—Since a subclass of AT$_2$ receptors sensitive to guanine nucleotides and PTX has been described in rat brain (21), the potential role of G protein-mediated inhibition of the T-type calcium current by AT$_2$ receptors was investigated by replacing intracellular GTP by the non-phosphorylable GDP analog, GDPβS, added through the patch micropipette. As a control, we determined the effects of GDPβS on [D-Ala^2,D-Leu^5]enkephalin (DADLE) inhibition of high voltage-activated calcium currents (L- or N-type), since it has been described that this inhibition is mediated by a PTX-sensitive G protein (31). Effective concentrations of GDPβS needed to prevent G protein modulation of calcium channels following G protein-coupled receptor activation range from 150 μM to 2 mM (32). In our cell system, addition of 3 mM GDPβS in the patch clamp pipette solution prevents the G protein-mediated inhibition of the high voltage-activated Ca^{2+} current by DADLE (46 ± 3%, $n = 14$) of peak current inhibition at 20 mV *versus* 9 ± 2% ($n = 5$) with GDPβS (data not shown). We found that intracellular application of 3 mM GDPβS also suppresses the AT$_2$-mediated inhibition of the T-type calcium current (5 ± 2% of decrease, $n = 9$) (Fig. 2, *A* and *B*). These results indicate that a GTPase activity mediates the AT$_2$ receptor inhibition of the T-type calcium channel. Moreover, addition of GTPγS, the non-hydrolyzable form of GTP, to the patch clamp pipette solution induces a slow decrease in current amplitude (30% ± 4, $n = 8$), which begins as soon as the patch is established (Fig. 2, *C* and *D*, *trace 2*). In experiments with GTPγS-filled pipette, addition of Ang II at the membrane break onset enhances the blocking effect of GTPγS on the T current by 51 ± 6% ($n = 6$ (data not shown). Moreover, Fig. 2, *C* and *D* (*trace 3*), shows that once the GTPγS-induced inhibition reaches a plateau, addition of Ang II produces an additional decrease in the T current amplitude.

Effect of PTX Treatment on Ca^{2+} Current Inhibition—To further characterize this G protein coupling, we verified whether the effect of Ang II was mediated via G_i or G_o proteins. Cells were incubated for 18 h with 100 ng/ml PTX to inactivate G_i and G_o by ADP-ribosylation. This treatment completely blocks the DADLE effect ($n = 9$) (data not shown), although the AT$_2$-mediated inhibition of the T-type calcium current is not significantly affected (20 ± 3% decrease by 100 nM CGP 42112 ($n = 9$) (Fig. 2 *E* and *F*)). However, this treatment results in complete ADP-ribosylation of G_i and G_o in NG108–15 cells (data not shown). We therefore conclude that neither G_i nor G_o are involved in the coupling mechanism between AT$_2$ receptors and T-type calcium channels.

Effect of GTPγS on Ang II Binding—As shown in Fig. 3*A*, competition binding experiments performed on plasma membranes prepared from NG108–15 cells that express only AT$_2$ receptor (26) show that addition of 1 mM GTPγS does not modify the Ang II inhibition of ^{125}I-labeled saralasin^1-isoleucine^8 binding, suggesting that the AT$_2$ receptor does not interact directly with a classical G protein.

Effect of Ang II on GTPase Activity—The effect of Ang II on GTPase activity was measured on plasma membranes prepared from non-differentiated NG108–15 cells. After activation, protein-bound GTP is hydrolyzed to GDP and inorganic phosphate by the intrinsic GTPase activity of the GTP binding protein itself, and the GTP-bound active conformation is converted to the GDP-bound inactive conformation (28). We found that both Ang II and CGP 42112 induced a 2.6-fold increase in the hydrolysis of [^{32}P]GTP in membrane fractions prepared from non-differentiated NG108–15 cells (Fig. 3*B*). The AT$_1$ selective antagonist DUP 753 (1 μM) does not affect GTPase activity, confirming the absence of AT$_1$ receptors in these cells. In contrast, the AT$_2$ antagonist PD 123319 (1 μM) reverses this stimulating effect (Fig. 3*B*).

Phosphotyrosine Phosphatase Activity Mediates the Effect of the AT$_2$ Receptor

Effect of Phosphotyrosine Phosphatase Inhibitors on the T-type Ca^{2+} Current Inhibition—Sodium orthovanadate at concentrations ranging from 50 to 100 μM is considered as a specific inhibitor of tyrosine phosphatases (33–35) while microcystin-LR completely and selectively inhibits Ser/Thr phosphatases of type 1 and 2A at concentrations higher than 5 nM (36). These inhibitors have been extensively used to inhibit either tyrosine or Ser/Thr phosphatases in many hormonal systems, including Ang II (20, 24, 37). As shown in Fig. 4*A*, 20 nM microcystin-LR in the patch micropipette does not modify the effect of 100 nM Ang II on the T-type calcium current (21 ±

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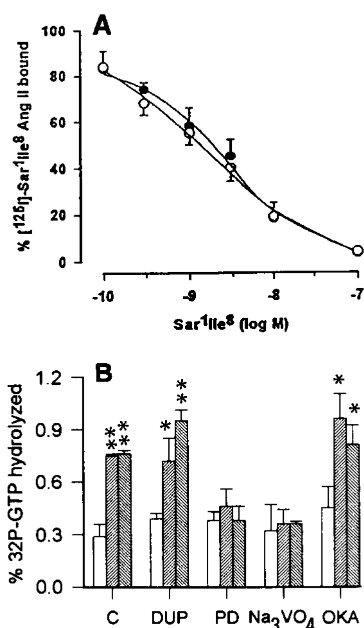

FIG. 3. **A G protein is involved in the AT_2 effect.** *A*, competition for Sar^1Ile^8 angiotensin II binding (where Sar is saralasin). Plasma membranes from NG108–15 cells (50 μg) were incubated with 0.2 nM ^{125}I-labeled Sar^1Ile^8-Ang II for 15 min at 37 °C in the presence of increasing concentrations of Ang II alone (●) or with 100 μM GTPγS (○). Results represented the mean ± S.E. from three different experiments, each conducted in triplicate incubations. *B*, effects of Ang II (100 nM) and CGP 42112 (100 nM) on the low K_m GTPase activity in NG108–15 cells. Pharmacological effects have been studied by the addition of DUP 753 (1 μM), a specific antagonist of the AT_1 receptor subtype, and PD 123310 (10 μM), a specific antagonist of the AT_2 receptor subtype. Involvement of phosphatase activity has been studied by the addition of sodium orthovanadate (Na_3VO_4, 100 μM), a tyrosine phosphatase antagonist, and okadaic acid (*OKA*, 1 mM), a Ser/Thr phosphatase antagonist. GTPase activity was determined by measuring the release of ^{32}P from [γ-^{32}P]GTP using 20 μg of membrane protein prepared from NG108–15 cells as described under "Materials and Methods." The low K_m GTPase activity was measured after subtracting the amount of hydrolysis that remained in the presence of 50 μM GTP (high K_m GTPase activity) from that measured with 0.03 μM [γ-^{32}P]GTP. The high basal K_m activity was not affected by addition of Ang II or CGP 42112. □, control (*C*); ▨, Ang II (100 nM); ▧, CGP (100 nM). Results are the mean ± S.E. of triplicate determinations from one experiment representative of three. **, $p < 0.001$; *, $p < 0.05$ (difference compared to control values).

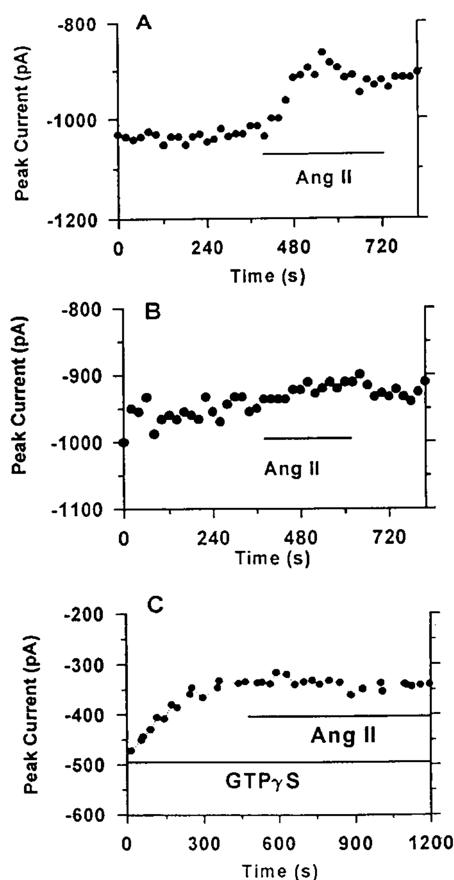

FIG. 4. **Involvement of a protein tyrosine phosphatase in AT_2 receptor signaling pathway.** *A*, 20 nM microcystin-LR (a specific Ser/Thr phosphatase 1 and 2A inhibitor) was added into the standard intracellular solution before application of Ang II. This addition did not alter the Ang II-mediated inhibition of the T-type Ca^{2+} current. *B*, 50 μM sodium orthovanadate (a specific tyrosine phosphatase inhibitor) added into the intracellular solution before application of Ang II abolished the Ang II-mediated inhibition of the T-type Ca^{2+} current. *C*, preincubation of cells in a solution containing sodium pervanadate (0.2 mM, 5 min) did not affect the blocking effect of intracellular GTPγS. At the plateau level, addition of Ang II did not produce any further blocking effect.

2% decrease, $n = 9$), whereas 50 μM sodium orthovanadate abolishes the Ang II-induced decrease of this current (6 ± 2% decrease, $n = 7$) (Fig. 4*B*). A PTPase monoclonal antibody (10 μg/ml) added to the pipette medium yields similar results (data not shown). These data clearly demonstrate that a phosphotyrosine phosphatase rather than a Ser/Thr phosphatase of type 1 or 2A is implicated in the coupling mechanism between AT_2 receptors and T-type calcium channels.

Effect of Phosphotyrosine Phosphatase Inhibitors on GTPase Activity Induced by Ang II or CGP 42112—As shown in Fig. 3*B*, when sodium orthovanadate (100 μM) is added together with Ang II or CGP 42112, the stimulation effect induced by the hormone alone is abolished. In contrast, addition of okadaic acid (1 mM), a blocker of Ser/Thr phosphatases, does not alter GTPase activity induced by Ang II or CGP 42112. In addition, these results indicate that all the elements of the signaling cascade are situated in the membrane fraction.

Sequential Events in the Signaling Pathway—Our results show that a PTPase and a G protein are involved in the modulation of the T current amplitude by the AT_2 receptor. To resolve the time sequence, we performed the following experiments. NG108–15 cells were preincubated for 5 min in a medium containing sodium pervanadate (0.2 mM) to block PTPase activity. After washout of pervanadate, the T current was recorded with a GTPγS-containing patch clamp pipette. Fig. 4*C* shows that the current amplitude decreases and reaches a plateau (36 ± 6% decrease, $n = 4$). Addition of Ang II at the plateau level does not produce any additional decrease.

The lack of additional effect of Ang II once the plateau is reached following GTPγS-induced blockage indicates that with sodium pervanadate preincubation, Ang II does not produce any further decrease of the T current because the AT_2 receptor is uncoupled from the G protein by sodium pervanadate inhibition of the PTPase (Fig. 4*C*). Furthermore, without sodium pervanadate preincubation, Ang II is able to further increase the blocking effect of GTPγS because the signaling pathway is intact (Fig. 2, *C* and *D*). These results indicate that PTPase activation is proximal to the AT_2 receptor activation.

Effect of Ang II on Protein Tyrosine Phosphorylation—Since the effect of Ang II on phosphorylation patterns varies according to the experimental model (10, 14, 17, 19, 20), we investigated this effect on the non-differentiated NG108–15 cells. With culture conditions used for Western blot experiments (1 × 10^6 cells/Petri dish), the endogenous Ang II production (38) could interfere with subsequent Ang II addition. For this reason, cells were preincubated overnight with lisinopril (an angiotensin-converting enzyme inhibitor) (38). Application of 100

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nM Ang II for 15 min decreases the level of tyrosine phosphorylation of a number of proteins, with a predominant effect on proteins of low molecular mass (20–22 kDa) (data not shown). The effect is specific since preincubation with PD 123319 reverses the Ang II effect.

The AT_2 receptor has recently been cloned and belongs to the class of the seven-transmembrane domain receptors (24, 25) along with somatostatin SSTR1 and dopamine D_3 receptors (39, 40). Using cDNA libraries from different sources (PC 12W and whole rat fetus), the two groups (24, 25) found similar amino acid sequences but opposite results concerning G protein involvement and phosphotyrosine phosphatase implication. The binding of ligand to the two cloned AT_2 receptors is not affected by GTPγS (24, 25). However, sensitivity to PTX and GDPβS indicates that one of these receptors involves a G protein in its signaling pathway (20, 25). This discrepancy could be explained by the presence of two subclasses of AT_2 receptors (6). Comparatively, we found that the native AT_2 receptor from NG108–15 cells demonstrates GTPγS-insensitive binding of Ang II and involvement of a GTPase activity in the transduction mechanism (Fig. 3).

Using the cell line PC12 W, Kambayashi *et al.* (25) and Takahashi *et al.* (20) demonstrated that the AT_2 receptor is linked to the inhibition of a PTPase via a G protein sensitive to pertussis toxin. However, in the same cell line, Bottari *et al.* (14) and Brechler *et al.* (17) demonstrated a stimulation of PTPase activity, which is independent of a G protein activation. When NG108–15 cells were treated overnight with lisinopril to block endogenous Ang II production (38, 41), we observed that Ang II decreases the amount of tyrosine phosphorylation of several proteins, which may be explained by stimulation of endogenous PTPase activity. This effect is abolished by coincubation with PD 123319, indicating its specificity toward the AT_2 receptor. These results and those of patch clamp experiments support the conclusion that a PTPase activity is involved in the inhibitory effect of Ang II on the T-type Ca^{2+} current, since orthovanadate and a PTPase monoclonal antibody reverse the effect of Ang II on this inhibition. Moreover, patch clamp and GTPase experiments demonstrate that a G protein is involved in this inhibition and that its activation is blocked by a tyrosine phosphatase antagonist. Although the signaling pathway needs to be determined more accurately, our results do allow us to propose the following time sequence. Activation of the AT_2 receptor is followed by activation of a PTPase, which is necessary for the coupling between a G protein and the T-type channel. In the present work, the exact substrate of PTPase has not been determined, but we can postulate that the coupling between the AT_2 receptor and the G protein is dependent upon the phosphorylated state of one of the components of the signaling pathway, *i.e.* the AT_2 receptor itself, the G protein, or an unknown accessory protein. Modulation of the coupling could thus be obtained by a balance between phosphatase and kinase activities. Association of a PTPase activity with activation of several seven-transmembrane domain receptors has been described either via a stimulation for somatostatin receptor (39, 42), dopaminergic D_2 receptor (34), and AT_2 receptor (14, 17, this paper) or via an inhibition (19, 20, 25). These effects may be direct (39, 40) or mediated via a PTX-sensitive G protein (34, 42).

In summary, the present observations demonstrate that in non-differentiated NG108–15 cells, which exclusively express AT_2 receptors, Ang II (100 nM) and CGP 42112 (100 nM) both decrease the T-type calcium current and that this effect is mediated by the involvement of a tyrosine phosphatase and a yet unknown G protein. Considering the abundance of T-type Ca^{2+} channels in neurons from fetal brain (43), the crucial role of Ca^{2+} in neuronal differentiation, and the abundance of AT_2 receptors during this period, it could be postulated that Ang II, via the AT_2 receptor, modulates the Ca^{2+} channel regulating pacemaker activity in neuronal cells. These modulations may have a profound effect on neuronal orientation, guidance, and differentiation (44).

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