In the present study, 3-day treatment of nondifferentiated NG108-15 cells with 100 nM angiotensin II (Ang II) induces morphological differentiation of neuronal cells characterized by the outgrowth of neurites. These morphological changes are correlated with an increase in the level of polymerized tubulin and in the level of the microtubule-associated protein, MAP2c. Mediation by the AT2 receptor may be inferred since: (a) these cells contain only AT2 receptors; (b) the effects are mimicked by CGP 42112 (an AT2 receptor agonist); (c) they are not suppressed by the addition of DUP 753 (an AT1 receptor antagonist); and (d) are abolished by co-incubation with PD 123319 (an AT2 receptor antagonist). Application of Ang II in dibutyryl cAMP-differentiated cells (which contain both types of receptors) induces neurite retraction, an effect mediated by the AT1 receptor. These results indicate that the AT1 receptor of Ang II induces neuronal differentiation, which is initiated through an increase in the levels of MAP2c associated with tubulin. Moreover, our results demonstrate that the AT1 receptor inhibits the process of differentiation induced by dibutyryl cAMP, whereas the AT2 receptors potentiate this effect, illustrating negative cross-talk interaction between the two types of Ang II receptors.

Pharmacological studies have clearly identified two classes of angiotensin II (Ang II) receptors. The AT1 receptor is closely associated with cardiovascular regulation, fluid volume homeostasis, and cellular growth (1, 2). Activation of the AT2 receptor is linked to phospholipase C activation and Ca\(^{2+}\) influx, effects which are mediated by G proteins (1, 2). The AT2 receptors have been identified in many fetal tissues, including brain (3–9), and in cell lines of neuronal origin (10–12). This receptor has been cloned (13, 14), but a definitive physiological function has yet to be assigned. We have reported previously that Ang II decreases the T-type calcium current in nondifferentiated NG108-15 cells expressing only the angiotensin AT2 receptor subtype (15, 16). Kang et al. (17) also reported that activation of the AT2 receptor increased a potassium channel activity. Moreover, Xiong and Marshall (18) reported that Ang II, via the AT2 receptor, could inhibit membrane depolarization and action potential elicited by N- methyl-D-aspartate receptors in the locus coeruleus, a brain area containing only Ang II receptors of the AT2 subtype. Considering the abundance of T-type Ca\(^{2+}\) channels in neurons from fetal brain (19), the crucial role of Ca\(^{2+}\) in neuronal differentiation (20) and the abundance of AT2 receptors during this developmental period, it could be postulated that Ang II, via the AT2 receptor, could affect some aspects of neuronal differentiation.

Neuronal differentiation is characterized by neurite extension that involves several biochemical steps directed toward promotion of the assembly of tubulin monomers into microtubules necessary to support the growing neurites. Several molecules play crucial roles in neurite outgrowth. Among these are the microtubule-associated proteins (MAPs) (21), which include both high molecular weight proteins, termed MAP1 to MAP5, and low molecular weight proteins, including tau (22). These proteins promote tubulin polymerization as well as stabilize microtubules and occur as embryonic and adult isoforms whose differential expression during brain development correlates with the maturation of neuronal circuitry. For example, MAP2 and tau bind to distinct populations of microtubules in adult neurons: MAP2 to somatodendritic microtubules and tau to axonal microtubules (23, 24). Expression of specific brain MAPs is critical for regulating neurite outgrowth and differentiation. Several lines of evidence have demonstrated a strong correlation between the pattern of expression of neuronal MAPs and the morphological differentiation of neurons (22).

In their nondifferentiated state, the hybrid cells NG108-15 (neuroblastoma × glioma) are rounded and actively dividing. Chronic exposure of NG108-15 cells to dibutyryl cAMP (db-cAMP) induces a process of differentiation that includes elaboration of neurites, development of electrical excitability, formation of functional synapses, alteration of ligand-gated channel properties, and a reduced rate of cell division. Thus, differentiated NG108-15 cells exhibit a neuronal phenotype, whereas glial properties appear to be suppressed (25, 26). Moreover, when nondifferentiated, NG108-15 cells express exclusively the AT1 receptor subtype and mainly the T-type Ca\(^{2+}\) channel (15). These cells also express tau and MAP2 and are thus useful in examining factors initiating neuronal differentiation.
Cell-bound radioactivity was separated by filtration as explained under Ang II (Fig. 1). For differentiation, the cells were cultured for 24 h in the absence (A, B) or in the presence of 100 nM Ang II (C, D), nondifferentiated cells (A, C) (cultured in DMEM containing 10% FBS) and differentiated cells (B, D) (cultured in a medium containing 1% FCS and 1 mM dibutyryl cAMP) were processed for competition binding experiments. Cells were incubated with 0.1 nM [3H]-[Sar1,Ile8]Ang II for 45 min at room temperature in the presence of increasing concentrations of Ang II (.), DUP 753 (.), CGF 42112 (.), or PD 123319 (.). Cell-bound radioactivity was separated by filtration as explained under “Materials and Methods.”

**Differentiation (27).** We have taken advantage of these properties to investigate the role of Ang II on differentiation and neurite outgrowth, with emphasis on cytoskeletal proteins. We found that Ang II induces outgrowth of neurites in nondifferentiated cells. These morphological changes are correlated with an increase in polymerized tubulin and an increase in the level of microtubule-associated MAP2c. In contrast, during the process of differentiation by dbcAMP, both AT1 and AT2 receptors are present, application of Ang II induces neurites involution. These results are the first to assign a physiological role for the AT2 receptor in neuronal differentiation, which is to induce neurite outgrowth in nondifferentiated cells (27).

**Materials and Methods**

**Cell Culture—**NG108-15 cells (provided by Drs. M. Emerit and M. Hamon; INSERM, U. 238, Paris, France) were cultured (passage 4 to 17) in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Inc., Burlington, Canada) with 10% fetal bovine serum (FBS; Life Technologies, Inc.). HAT supplement (hypoxanthine, aminopterin, thymidine) (Life Technologies, Inc.), and 50 mg/liter gentamicin at 37°C in 80-cm² Nunclon Delta flasks in a humidified atmosphere of 95% air and 5% CO₂, as suggested by Hamprecht et al. (26). The medium was replaced every 2 days. Subcultures were performed at confluence. Under these conditions, cells express only the AT1 receptor subtype of Ang II (Fig. 1A). For differentiation, the cells were cultured for 24 h in the normal culture medium and thereafter replaced by DMEM low glucose (1 mg/liter glucose) containing 1% fetal calf serum (Inotech), HAT supplement, 50 mg/liter gentamicin, and 1 mM dbcAMP (Boehringer Mannheim, Montreal, Canada). The cells were allowed to differentiate for 24 h prior to Ang II or Ang II/analogs treatment. Cells were cultured for 3 subsequent days under these conditions, with the differentiation medium being changed every day. For all experiments, cells were plated at the same initial density. Experiments and cytoskeleton extractions were performed on the 4th day.

**Binding Studies—**The analog [Sar1,Ile8]Ang II was iodinated by the iodogen method and separated on a 25-cm C-18 µ-Bondapak column (Waters, Milford, MA) with a linear gradient of 20–60% acetonitrile in a buffer of 7% isopropanol, 0.25 M ammonium acetate, pH 5.0, at a flow rate of 1 ml/min. Carrier-free moniodinated product was obtained as a single homogeneous peak at 20 min. The specific activity was approximately 1000 Ci/mmol. Binding assays were performed on cultured cells as described previously (28). NG108-15 cells (1.0–1.5×10⁶ cells/Petri dish) were washed with 2 ml of Hank's buffered saline (HBS: NaCl, 130 m; KCl, 3.5 m; CaCl₂, 1.8 m; MgCl₂, 0.5 m; NaHCO₃, 2.5 m; HEPES, 5 mM, supplemented with 1 g/liter glucose and 0.5% BSA) and incubated for 15 min at 22°C in the same medium. The hormone binding reaction was initiated by quick aspiration of the HBS medium and addition to each Petri dish of 0.8 ml of HBS containing the labeled peptide alone or with analogs as described above. Incubations were performed in triplicates for 45 min at room temperature (22°C). After incubation, cells were rapidly detached by scraping with a rubber policeman. Incubations media were filtered through Whatman GF/C filters. Filtrates were counted in a Beckman standard liquid scintillation system equipped with a 10 ml counting vial of scintillation fluid. Specific binding was calculated as the displacement curve illustrated in Fig. 1. It shows that, in nondifferentiated cells, DUP 753 did not displace the iodinated Ang II analog from the Ang II receptor in cell lines (Fig. 1A), although, in differentiated cells, labeling was displaced by DUP 753 (Fig. 1B), indicating the presence of AT1 receptors. These results show that nondifferentiated cells contain only AT2 receptors, while cells that have initiated differentiation express both subtypes of the Ang II receptor. Three days of Ang II treatment induced the expression of AT1 receptors, which remained in low proportions however (20% displacement) compared with the AT2 receptors (Fig. 1C), while decreasing the number of AT2 receptors in dbcAMP-differentiated cells (20% displacement compared with 50% in control dbcAMP-treated cells) (Fig. 1D).

**Preparation of Microtubule Proteins—**Preparations enriched in microtubules were obtained from cells grown in 100-mm Petri dishes as described by Solomon (29) with some modifications. The cells were pretreated with 1 μM Taxol (Sigma) for 2 h before extraction of microtubules. At this concentration Taxol stabilizes microtubules without promoting polymerization. The culture medium was then aspirated and replaced by PM2 buffer (PIPES, 0.1 m; glycerol, 2 m; MgCl₂, 5 m; EGTA, 2 mM, aprotinin, 40 TIU/ml; phenylmethylsulfonyl fluoride, 2 mM; benzamidine, 1 mM, pH 6.9) containing Taxol (1 μM). Cells were scraped from the substratum with a rubber policeman and transferred to a 15-mL conical tube and centrifuged at 1000 × g for 5 min at 37°C. The cell pellet was then extracted with PM2 buffer containing 1% Nonidet P-40 and 1 μM Taxol. After a 15-min incubation on ice, the suspension was centrifuged at 1000 × g for 5 min at 37°C. The cell pellet containing the microtubules and associated proteins was then solubilized in Tritis buffer 125 mM, pH 6.8, containing 4% sodium dodecyl sulfate (SDS) (w/v), 20% glycerol (w/v), and 10% β-mercaptoethanol (w/v) and heated to 100°C for 5 min. After centrifugation at 10,000×g for 5 min, the supernatant was stored at −20°C until Western blot analysis. For total cell extracts, cells grown in 100-mm Petri dishes were washed twice with HBS buffer and solubilized as described above.

**Western Blotting—**β-Tubulin was detected with monoclonal antibody purchased from Amersham (Oakville, Ontario, Canada); monoclonal antibody that recognizes all MAP2 forms was purchased from Sigma and the monoclonal tau antibody SE2 was kindly provided by Dr. Ken Kosik (Center for Neurologic Diseases, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA). Samples from equivalent number of cells were compared in each experiment. Samples were separated on 4–15% (w/v) SDS-polyacrylamide gels. Proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA). Membranes were blocked with 1% gelatin, 0.05% Tween 20 in TBS buffer, pH 7.5. After three washes with TBS/Tween 20 (0.05%), membranes were incubated either with anti-tubulin (1:250), anti-MAP2 (1:500), or anti-tau (1:750) for 2 h at room temperature, followed by four washes with TBS/Tween 20. Detection was accomplished using horseradish peroxidase-conjugated anti-mouse antibody (Amersham, Oakville, Ontario, Canada) and enhanced chemiluminescence (ECL; detection system Amersham). The immunoreactive bands were scanned by laser densitometry and expressed in arbitrary units.

**Immunofluorescence—**For immunofluorescence, cells were plated on plastic coverslips (Starsted, St. Laurent, Quebec, Canada) and were grown and treated for 3 days with appropriate stimulus. Cells were rinsed twice with HBS, fixed with formaldehyde 3.7% (w/v) (in HBS), pH 7.4, and permeabilized with 0.2% Triton X-100. Cells were then incubated for 1 h at room temperature with the monoclonal MAP2 antibody (1:400), monoclonal tubulin antibody (1:1000), and the monoclonal tau antibody 5E2 was kindly provided by Dr. Ken Kosik (Center for Neurologic Diseases, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA). After three washes with TBS/Tween 20 (0.05%), the coverslips were blocked with 5% normal goat serum in TBS for 1 h at room temperature. Cells were then incubated for 1 h at room temperature with the primary antibody: anti-MAP2 (1:500), anti-tubulin (1:250), anti-MAP2 (1:500), or anti-tau (1:750) for 2 h at room temperature, followed by four washes with TBS/Tween 20. Detection was accomplished using horseradish peroxidase-conjugated anti-mouse antibody (Amersham, Oakville, Ontario, Canada) and enhanced chemiluminescence (ECL; detection system Amersham). The immunoreactive bands were scanned by laser densitometry and expressed in arbitrary units.
AT_2 Receptor Induces Neurite Outgrowth in NG108-15 Cells

**RESULTS**

**Morphological Effects Induced by Ang II on NG 108-15 Cells**

We first examined the effects of Ang II on the morphological changes and cytoskeletal interactions that lead to the initial neurite outgrowth from neuronal cell bodies. Cells were plated at a density of 5 x 10^4 cells/dish. As shown by phase-contrast microscopy, after 3 days in culture, nontreated, nondifferentiated control cells were actively dividing and had round cell bodies, although some cells exhibited thin processes (Fig. 2A). After a 3-day treatment with Ang II (100 nM), most cells extended one or two neurite processes with a growth cone at their tip (Fig. 2B) along with a longer process, while the cell body retained a rounded appearance. Moreover, the number of cells was lower than in control, nontreated cells (from 4.3 ± 0.8 x 10^5 cells/control dishes to 3.2 ± 0.6 x 10^5 cells/Ang II-treated dishes, 25% decrease), indicating that the rate of cell division had decreased. Cell viability was not affected since they remained attached to the substratum, persistently excluded had decreased. Cell viability was not affected since they remained attached to the substratum, persistently excluded. The coverslips were then mounted in Vectashield (Vector Laboratories, Burlingame, CA).

In order to quantify and to better characterize the pharmacology of the effects mediated by Ang II, the amount of polymerized tubulin was analyzed in detergent-extracted cytoskeletal fractions prepared from control, Ang II- or Ang II/analog-treated cells. Since neurite outgrowth requires large amounts of tubulin, its measurement represents an index of neurite extension. Cytoskeletal fractions from equivalent numbers of cells were analyzed in parallel. Thus, the cellular contents of each of the major proteins could be compared directly. As shown in Fig. 6A, in nondifferentiated cells treated for 3 days with Ang II, the level of tubulin had increased compared with nontreated control cells (lane 2 versus lane 1). The effect was specific to the AT_2 receptor, since co-incubation with 10 μM PD 123319, a specific AT_2 antagonist,
reversed the effect of Ang II (lane 3 versus lane 1), whereas co-incubation with 10 μM DUP 753, which inhibits AT₁ receptor, did not alter tubulin levels induced by Ang II (lane 4 versus lane 2). Moreover, 3-day treatment with 100 nM CGP 42112 (an AT₂ receptor agonist) produced an effect similar to Ang II (lane 5 versus lane 2). Comparative densitometric analysis from five different experiments are illustrated in Fig. 7A and show that Ang II and CGP 42112, respectively, induced a 7- and 6-fold increase of tubulin levels compared with nontreated control cells and conclusively demonstrate that the effect is mediated through the AT₂ receptor. Changes in microtubule levels may be due to an increase in total cell tubulin content or may reflect increased polymerization of the tubulin pool. In order to verify this point, total tubulin levels were measured in cellular extracts. As shown in Fig. 6B, Ang II did not induce significant changes in the level of total tubulin content.

MAP2 and Tau Levels—Since MAPs affect microtubule assembly, stability, and cross-linking in developing brain (22), MAP2 and tau levels were characterized and compared in nondifferentiated control, Ang II- and Ang II/analog-treated cells. A monoclonal antibody recognizing a common epitope in both adult (MAP2a and -b) and juvenile (MAP2c) (27, 30) forms was used in Western blots to assess protein levels of MAP2c in actively dividing and in differentiated cells. Fig. 6C shows that the antibody to MAP2 revealed two bands with similar intensities of around 85 and 76 kDa. Three-day treatment with Ang II induced a 5.3-fold increase in both MAP2c bands (Fig. 6C, lane 2 versus lane 1 and Fig. 7B). This effect was reproduced with CGP 42112 (4.7-fold increase) (Fig. 6C, lane 5 versus lane 1 and Fig. 7B) and reversed when Ang II was co-incubated with PD 123319 (lane 4 versus lane 2). However, addition of DUP 753, the AT₁ receptor antagonist, also inhibited the Ang II effect (Fig. 6C, lane 3 versus lane 2 and Fig. 7B).

Tau was detected with the monoclonal antibody S5E2. In nondifferentiated cells, tau appeared as a single isoform with a molecular mass of 58 kDa (Fig. 6D). The level of tau was not significantly affected by treatment with Ang II or Ang II/analogs.

Effect of Ang II on Tubulin and Microtubule-associated Proteins in Differentiated Cells

Tubulin Levels—As equal amount of cells were analyzed by Western blotting, we could observe that the level of tubulin had...
AT₂ Receptor Induces Neurite Outgrowth in NG108-15 Cells

Ang II Effects in Nondifferentiated Cells

Ang II Increases the Level of Microtubule-associated-MAP2c, but Not Microtubule-associated Tau—The addition of Ang II to nondifferentiated cells increased the level of MAP2c associated with tubulin, which may directly affect its polymerization. This effect is mediated by the AT₂ receptor, since it is reproduced by CGP 42112. Moreover, when nondifferentiated cells were co-incubated with Ang II and PD 123319, they maintained their rounded appearance, without any outgrowth processes, similar to control cells. In addition, Ang II or Ang II/analog treatments do not affect the amount of tau protein associated with microtubules. Microtubule-associated proteins play a pivotal role in the process of neurite elongation (22). For example, suppression of MAP2 expression by means of antisense oligonucleotides inhibits the outgrowth of exploratory neurites in cultured cerebellar neurons (38), whereas further neurite elongation depends upon tau (39). Therefore, our observations indicate that Ang II has a specific effect on the initial establishment of neurites, without affecting axonal elongation. Specific MAPs are also indicators of the state of brain differentiation since most exist in juvenile and mature isoforms. For instance, MAP2c is the juvenile form and MAP2a is one of the mature forms, while MAP2b is present throughout rat brain development (22). In the present experiments, the MAP2 antibody detected two polypeptides in Western blots of nondifferentiated NG108-15 cells at 76 and 85 kDa. These two bands could
correspond to two different states of phosphorylation of MAP2c. Alternatively, they could also represent the two spliced isoforms of MAP2c (40). Indeed, MAP2c exists as two isoforms with either three or four tubulin binding domains, the three domain form being mainly expressed in neurones, and the four domain form being mainly expressed in glial cells (40). Both isoforms may be expressed in NG108-15 cells, due to their neuroblastoma × glial hybrid origin. The specific increase of the lower molecular weight form in response to neuronal differentiation induced by dbcAMP (Fig. 6C, lane 1 versus Fig. 2C, lane 1) would favor this hypothesis and further indicate that Ang II stimulates differentiation of NG108-15 cells along a neuronal pathway. Beamann-Hall and Vallano (27) observed the presence of high molecular weight forms of MAP2, MAP2a, and MAP2b, after 9 days of differentiation, whereas we did not after 3 days. It may be that, under the experimental conditions used by these authors, cells have already achieved complete neuronal differentiation, as indicated by the presence of only one MAP2c isoform in their undifferentiated state.

Consequence of the Ang II-induced AT1 Receptors Expression—As shown in Fig. 1C, 3-day treatment with Ang II induces expression of AT1 receptors which anyway remain lower compared with the AT2 receptors. The presence of these receptors may explain the discrete morphological differences observed between Ang II- and CGP 42112-treated cells as well as higher levels of MAP2 associated with microtubules. The effects observed with CGP 42112 appear stronger than those observed with Ang II. In particular, CGP 42112-treated cells exhibit several short dendrites. These differences may be due to the presence of AT1 receptors seen after the 3-day treatment with Ang II, which may counteract the effect of the AT2 receptor. Although DUP 753 did not affect tubulin levels, it decreased MAP2 expression as did PD 123319, suggesting that both receptors may be involved in the regulation of MAP2 associated with tubulin. Nevertheless, the expression of the AT1 receptor is low and we cannot rule out the possibility that the inhibitory effect of DUP 753 on MAP2c expression may be nonspecific. Such nonspecific effects of DUP 753 have been shown previously in other systems (41, 42). Collectively, our results support the hypothesis that Ang II, via the AT2 receptor, induces neurite outgrowth, by acting specifically on the increased association of MAP2 with tubulin.

Ang II Effects in dbcAMP-differentiated Cells

NG108-15 cells differentiated with dbcAMP exhibit a neuronal phenotype characterized by a long axon-like process and one or two short dendrite-like processes. These morphological observations are correlated with the increased expression of tau proteins (Fig. 6, H versus D, lane 1) and MAP2 (Fig. 6, G versus C, lane 1). Application of Ang II during this differentiating period induced a rounding appearance of the cells, which still maintained their long axon-like process (Figs. 3B versus 2C). These observations further indicate that the target of Ang II effect is MAP2 rather than tau. This inhibitory effect is mediated by activation of the AT1 receptor, since it is not modified by co-incubation with PD 123319 (data not shown), but abolished if cells are co-incubated with DUP 753 (Fig. 3, C versus 3A). Moreover, if dbcAMP-treated cells are incubated with CGP 42112, an effect mediated by the AT2 receptor is observed (cells exhibit several processes). Western blot experiments support these morphological data, since co-incubation with Ang II + DUP 753 (Fig. 6E, lane 3 versus lane 1) induces an important increase in tubulin level similar to that observed in nondifferentiated cells treated with Ang II (Fig. 6A, lane 2 versus 1). These results indicate that activation of the AT2 receptor inhibits the process of differentiation induced by dbcAMP (at least on the short processes). Similar observations have been reported for dopamine receptors. Both D1 and D2 receptors are present in fetal brain. While stimulation of the D2 receptor increased branching and extension of neurites (43), stimulation of the D1 receptor reduced neurite outgrowth (44), suggesting that hormones and neurotransmitters may be capable of controlling the development of specific types of neurones. Moreover, Ang II treatment of dbcAMP-differentiated cells decreases the number of AT1 receptors. This observation that Ang II down-regulates its own receptor has been previously described in other cell types (45). This indicates that Ang II induced a time-dependent expression of the AT1 receptor which obviously exerts a fine modulation of AT2 receptor-induced neurite outgrowth.

What Could Be the Mechanism of Action of Ang II on MAP2 and Tubulin Polymerization?

The mechanism by which Ang II, via the AT2 receptor, increases the amount of MAP2c associated with tubulin and, as a likely consequence, tubulin polymerization, remains to be elucidated. However, two types of mechanisms can be considered. 1) A possible effect of Ang II on MAP2 and tubulin state of phosphorylation. Accumulating data demonstrate that the AT2 receptor is associated with stimulation of a tyrosine phosphatase activity (46, 47). Preliminary results by our group (16, 48) have shown that the AT2 receptor modulates phosphorylation of tyrosine residues of several proteins, with a predominant effect on proteins with molecular masses of 110–120, 72–75, and 20–22 kDa. A reduction in MAP kinase activity by the AT2 receptor has also been recently described in AT2 receptor-transfected cells (49). Moreover, we found that the AT2 receptor inhibits p21<sup>ras</sup> activity (48), which could be one of the first steps in the aforementioned reduction of MAP kinase activity (49). Activation of AT2 receptor by modulating the state of phosphorylation of MAP2c could provide an additional level of control of tubulin polymerization. Several studies have shown that phosphorylation of tubulin and MAP2 inhibited microtubule assembly (50), while dephosphorylation increased it (51). It could be suggested that, in control nondifferentiated cells, growth factors present in the culture medium may stimulate the phosphorylation of MAP2c, thus preventing its binding to tubulin. Ang II, via the AT2 receptor, could inhibit a phosphorylation cascade, in turn leading to MAP2c dephosphorylation, hence increasing its ability to interact with tubulin to promote microtubule assembly. 2) Involvement of calcium in cytoskeleton dynamics has also been largely reported (20). Based on our recent observations that the AT2 receptor inhibits the T-type Ca<sup>2+</sup> current (15, 16) and thereby modulates intracellular calcium concentration, we can postulate that this modulation could play a pivotal role in the process of AT2-induced polymerization-depolymerization of tubulin. This hypothesis is reinforced by the observation that the expression of T-type Ca<sup>2+</sup> channels are developmentally regulated, indicating their involvement in brain development (19).

The negative cross-talk between AT2 and AT1 receptors was observed at different levels. It has been demonstrated recently in vascular smooth muscle cells transfected with the AT2 receptor (52), and in endothelial cells (53), that the AT2 receptor has an antiproliferative effect and inhibits the growth action of the AT1 receptor. Ichiki et al. (54), using transgenic mice lacking the AT2 receptor, have shown that this receptor mediates a depressor effect and antagonizes the AT1-mediated pressor action of Ang II. All these data suggest that the AT1 receptor may be implicated in cell growth, in part via p21<sup>ras</sup> and MAP kinase activation (55), and that the AT2 receptor could initiate differentiation by inhibiting the same pathways as well.
AT₂ Receptor Induces Neurite Outgrowth in NG108-15 Cells

Taken together, our data suggest that Ang II, via the AT₂ receptor, promotes neurite outgrowth by increasing the amount of polymerized tubulin which likely results from an increased association of MAP2c with tubulin. Moreover, the presence of polymerized tubulin which likely results from an increased receptor, promotes neurite outgrowth by increasing the amount of tubulin. Therefore, the AT₁ receptor inhibits the process of differentiation induced by dbcAMP. The precise nature of this interaction remains to be elucidated.

Acknowledgments—We thank Lucie Chouinard for technical assistance, Dr. G. Guillemette for iodination of angiotensin II and Mylène Côté for assistance in taking fluorescence micrographs. We are also indebted to Dr. Ken Kosik for providing us with the tau 5E2 antibody and Dr. Andrew Matus for helpful discussions and suggestions on this work.

REFERENCES
1. Bottari, S. P., de Gasparo, M., Stockelings, U. M., and Levens, N. R. (1993) Front. Neuroendocrinol. 14, 123–171
2. Timmermans, P. B. M. W. M., Weng, P. C., Chiu, A. T., Herblin, W. F., Benfield, P., Carini, D. J., Lee, R. J., Wexler, R. R., Say, J. A. M., and Smith, R. D. (1993) Pharmacol. Rev. 45, 205–257
3. Gehlert, D. R., Gackenheimer, S. L., and Schober, D. A. (1991) Neuroscience 44, 501–514
4. Obermüller, N., Unger, T., Culman, J., Gohlke, P., De Gasparo, M., and Bottari, S. P. (1991) Neursci. Lett. 132, 11–15
5. Millan, M. A., Jacobowitz, D. M., Aguilera, G., and Catt, K. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1140–1144
6. Tsusumi, K., Striemenberg, C., Viswanathan, M., and Saavedra, J. M. (1991) Endocrinology 129, 1075–1082
7. Tsuchi, K., and Saavedra, J. M. (1992) Mol. Pharmacol. 41, 290–297
8. Barnes, J. M., Lucinda, J. S., Barber, P. C., and Barnes, N. M. (1993) Eur. J. Pharmacol. 230, 251–258
9. Heemskerk, F. M. J., Zorad, S., Seltzer, A., and Saavedra, J. M. (1993) J. Clin. Invest. 91, 1135–1143
10. Speth, R. C., Mei, L., and Yamamura, H. I. (1989) Pept. Res. 2, 232–239
11. Leung, K. H., Roscoe, W. A., Smith, R. D., Timmermans, P. B. M. W. M., and Inagami, T. (1993) Mol. Pharmacol. 43, 1614–1623
12. Speth, R. C., Mei, L., and Yamamura, H. I. (1989) Brain Res. Rev. 15, 101–120
13. Matus, A., Bernhardt, R., and Hughes-Jones, T. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3010–3014
14. Binder, L. I., Frankfurter, A., and Rehbn, L. I. (1985) J. Cell Biol. 101, 1371–1378
15. Hamprecht, B. (1977) Int. Rev. Cytol. 49, 99–170
16. Hamprecht, B., Glaser, T., Reiser, G., Bauer, E., and Propst, F. (1985) Methods Enzymol. 109, 316–341
17. Beaman-Hall, C. M., and Vallano, M. L. (1993) J. Neurobiol. 24, 1500–1516
18. Guillén, G., Gallo-Payet, N., Ballestre, M.-N., and Lombard, C. (1986) Biochem. J. 232, 765–775
19. Solomon, F. (1986) Methods Enzymol. 134, 139–147
20. Weisshaar, E., Doll, D., and Matus, A. (1992) Development (Camb.) 116, 1151–1161
21. Tallant, E. A., Diz, D. I., Khosla, M. C., and Ferrario, C. M. (1991) Hypertension 17, 1135–1143
22. Speth, R. C., Mei, L., and Yamamura, H. I. (1989) Pept. Res. 2, 232–239
23. Carrithers, M. D., Masuda, S., Koide, K. A., and Weyhenmeyer, J. A. (1992) Neuroscience 59, 4567–4571
24. Binder, L. I., Frankfurter, A., and Rebhun. L. I. (1985) J. Neurosci. 5, 3420–3426
25. Hamprecht, B., Glaser, T., Reiser, G., Bayer, E., and Propst, F. (1985) Methods Enzymol. 109, 316–341
26. Millan, M. A., Jacobowitz, D. M., Aguilera, G., and Catt, K. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1140–1144
27. Tsuchi, K., Striemenberg, C., Viswanathan, M., and Saavedra, J. M. (1991) Endocrinology 129, 1075–1082
28. Barnes, J. M., Lucinda, J. S., Barber, P. C., and Barnes, N. M. (1993) Eur. J. Pharmacol. 230, 251–258
29. Heemskerk, F. M. J., Zorad, S., Seltzer, A., and Saavedra, J. M. (1993) J. Clin. Invest. 91, 1135–1143
30. Speth, R. C., Mei, L., and Yamamura, H. I. (1989) Pept. Res. 2, 232–239
31. Leung, K. H., Roscoe, W. A., Smith, R. D., Timmermans, P. B. M. W. M., and Inagami, T. (1993) Mol. Pharmacol. 43, 1614–1623
32. Halama, L., Payet, M.-D., De Gasparo, M., and Gallo-Payet, N. (1994) Nature 369, 675–678
33. Carrithers, M. D., Masuda, S., Koide, K. A., and Weyhenmeyer, J. A. (1992) Neuroscience 59, 4567–4571
34. Binder, L. I., Frankfurter, A., and Rebhun. L. I. (1985) J. Neurosci. 5, 3420–3426
35. Hamprecht, B., Glaser, T., Reiser, G., Bayer, E., and Propst, F. (1985) Methods Enzymol. 109, 316–341
36. Millan, M. A., Jacobowitz, D. M., Aguilera, G., and Catt, K. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1140–1144
37. Takahasi, K., Bardhan, S., Kambayashi, Y., Shirai, H., and Inagami, T. (1994) Biochem. Biophys. Res. Commun. 198, 60–66
38. Fukunaga, K., and Dzau, V. J. (1993) J. Clin. Invest. 91, 11440–11444
39. Ullian, M. E., and Lina, S. L. (1989) J. Clin. Invest. 84, 840–846
40. Brechler, V., Reichlin, S., de Gasparo, M., and Bottari, S. P. (1994) Rept. Channels 2, 89–97
41. Kater, S. B., and Mills, L. R. (1991) J. Neurosci. 11, 891–899
42. Muller, N., Unger, T., Culman, J., Gohlke, P., De Gasparo, M., and Bottari, S. P. (1991) Neursci. Lett. 132, 11–15
43. Millan, M. A., Jacobowitz, D. M., Aguilera, G., and Catt, K. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1140–1144
44. Tsuchi, K., and Saavedra, J. M. (1992) Mol. Pharmacol. 41, 290–297
45. Barnes, J. M., Lucinda, J. S., Barber, P. C., and Barnes, N. M. (1993) Eur. J. Pharmacol. 230, 251–258
46. Heemskerk, F. M. J., Zorad, S., Seltzer, A., and Saavedra, J. M. (1993) Neurropept 4, 103–105
47. Speth, R. C., Mei, L., and Yamamura, H. I. (1989) Pept. Res. 2, 232–239
48. Webb, M. L., Liu, E. C.-K., Cohen, R. B., Hedberg, A., Bogosian, E. A., Monshizadegan, C. M., Serafino, R., Moreland, S., Murphy, T. J., and Dickinson, K. E. J. (1992) Peptides 13, 499–508
49. Leung, K. H., Roscoe, W. A., Smith, R. D., Timmermans, P. B. M. W. M., and Chiu, A. T. (1991) J. Pharmacol. Exp. Ther. 251, 258–264
Angiotensin II Induction of Neurite Outgrowth by AT₂ Receptors in NG108-15 Cells: EFFECT COUNTERACTED BY THE AT₁ RECEPTORS

Liette Laflamme, Marc de Gasparo, Jean-Marc Gallo, Marcel D. Payet and Nicole Gallo-Payet

J. Biol. Chem. 1996, 271:22729-22735.
doi: 10.1074/jbc.271.37.22729

Access the most updated version of this article at http://www.jbc.org/content/271/37/22729

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 55 references, 15 of which can be accessed free at http://www.jbc.org/content/271/37/22729.full.html#ref-list-1