Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4655–4659, July 1987
Neurobiology

Distinct angiotensin II receptor in primary cultures of glial cells from rat brain

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Communicated by Philip Teitelbaum, February 26, 1987

ABSTRACT **Angiotensin II (Ang-II) has profound effects on the brain. Receptors for Ang-II have been demonstrated on neurons, but no relationship between glial cells and Ang-II has been established. Glial cells (from the hypothalamus and brain stem of 1-day-old rat brains) in primary culture have been used to demonstrate the presence of specific Ang-II receptors. Binding of ^{125}I-Ang-II to glial cultures was rapid, reversible, saturable, and specific for Ang-II. The rank order of potency of ^{125}I-Ang-II binding was as follows: Ang-II = [sarcosine^1,Ala^8]Ang-II > [sarcosine^1,Ile^8]Ang-II >> Ang-III > Ang-I. Scatchard analysis revealed a homogeneous population of high-affinity (K_d = 1.1 nM) binding sites with a B_{max} of 110 fmol/mg of protein. Light-microscopic autoradiography of ^{125}I-Ang-II binding supported the kinetic data, documenting specific Ang-II receptors on the glial cells. Ang-II stimulated a dose-dependent hydrolysis of phosphatidylinositols in glial cells, an effect mediated by Ang-II receptors. However, Ang-II failed to influence [^{3}H]norepinephrine uptake, and catecholamines failed to regulate Ang-II receptors, effects that occur in neurons. These observations demonstrate the presence of specific Ang-II receptors on the glial cells in primary cultures derived from normotensive rat brain. The receptors are kinetically similar to, but functionally distinct from, the neuronal Ang-II receptors.**

The brain contains all the components of a renin–angiotensin system (1–4) and specific receptors for angiotensin II (Ang-II; ref. 5). Likewise, primary neuronal cultures prepared from rat brains exhibit specific Ang-II receptors, Ang-II, and Ang-II synthetic mechanisms (6–8). These neuronal cultures consist of 80–85% neuronal cells and 10–15% non-neuronal (glial) cells as evidenced by immunocytochemical staining (7). Kinetic and autoradiographic analyses have revealed a single population of Ang-II-specific receptors on neuronal cells, with a K_d value of ≈1 nM (8).

These neuronal Ang-II receptors are downregulated by catecholamines via α_1-adrenergic receptors (9, 10) and upregulated by protein kinase C agonists and mineralocorticoid hormones (11, 12). Neuronal Ang-II receptors modulate discrete functions; for example, Ang-II modulates catecholamine metabolism and levels (13–15) in neuronal cultures by acting via specific receptors. Therefore, a kinetically and functionally discrete population of Ang-II receptors appears to be associated with the neuronal cells.

However, in neuronal culture preparations in which the non-neuronal cell population has exceeded 10–15% of the total, the Ang-II-specific binding and physiological effects of Ang-II are more variable (8). Considering this observation and the fact that glial cells contain receptors for other peptide hormones (16), we tested the hypothesis that specific Ang-II receptors were present on glial cells. The results reported demonstrate that glial cells in primary culture, indeed, contain specific Ang-II receptors that are kinetically similar to, but functionally different from, Ang-II receptors on neuronal cells.

METHODS

Preparation of Primary Astrocytic Glial Cells in Culture. Glial cultures of hypothalamus and brain stem from 1-day-old Wistar-Kyoto rat brains were prepared as described (16). In brief, brains from 1-day-old rats were dissected and placed in an isotonic salt solution containing 100 units of penicillin G, 100 μg of streptomycin, and 0.25 μg of Fungizone per ml at pH 7.4. The brain stem (medulla oblongata and pons) was dissected free from the rest of the brain, and all blood vessels and pia mater were removed. A hypothalamic block containing the supraoptic, paraventricular, anterior, dorsomedial, ventromedial, lateral, and posterior nuclei was dissected from the remainder of the brain, and all blood vessels and pia mater were removed. The brain stem and hypothalamic pieces were combined and minced into ≈2-mm pieces; the brain pieces were suspended in trypsin, and the cells were dissociated by treatment with trypsin and DNase I (16).

Dissociated cells were suspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, washed once, and were finally suspended in the same medium at a concentration of 1×10^6 cells per ml. They were plated in 100-mm-diameter Falcon tissue culture dishes precoated with poly-L-lysine and incubated at 37°C in a humidified incubator with 5% CO^2/95% air. After 3 days in culture, the medium was replaced with fresh DMEM containing 10% fetal calf serum, and cultures were maintained at 37°C for an additional 3 days. At this time cultures were washed once with DMEM, and cells were dissociated by trypsin treatment. Dissociated cells suspended in DMEM containing 10% fetal calf serum were inoculated in Falcon tissue culture dishes at a density of 1×10^5 cells per 35-mm-diameter culture dish for the appropriate experiments, and cells were grown for 6–8 days at 37°C before use.

Phase-contrast microscopic examination of cultures on day 6–8 demonstrated confluent monolayers consisting of large, flat, light-appearing cells. Antibody to glial fibrillary acidic protein, a marker specific for astrocytic glial cell type, was used to determine the composition of glial cells prepared in this fashion. Ninety-five to 98% of the cells in primary culture were stained by this antibody, indicating that the majority of the cells in culture were of the astrocytic glial type. Fig. 1 shows positively stained cells as an example. No significant number of fibroblastic or oligodendrocyte cell types were observed in these cultures.

Analysis of ^{125}I-Ang-II Binding to Glial Cultures. The specific binding of ^{125}I-Ang-II to cell membrane receptors was determined in intact glial cells attached to 35-mm-diameter tissue culture dishes, as described (8, 9). Briefly,

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Abbreviations: Ang-, angiotensin; P_i/NaCl, Dulbecco's phosphate-buffered saline; BSA, bovine serum albumin; NE, norepinephrine; Sar, sarcosine; DMEM, Dulbecco's modified Eagle's medium. B_{eq}, bound ^{125}I-Ang-II at equilibrium.

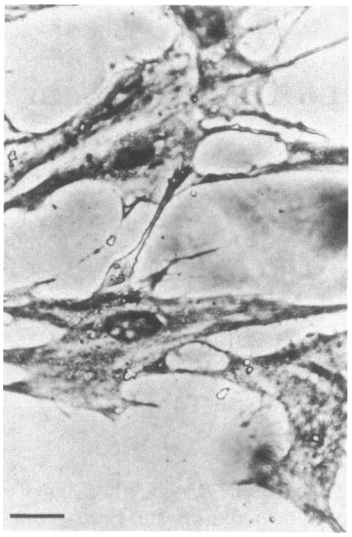

FIG. 1. Staining of glial cultures with anti-glial fibrillary acidic protein (GFAP) antibody by peroxidase-antiperoxidase (PAP) method. Glial cultures were fixed, incubated with a 1:100 dilution of anti-GFAP antibody for 24 hr at 4°C, and used for staining by the PAP method. Bar = 10 μM.

growth medium was removed from the dishes, cultures were washed with Dulbecco's phosphate-buffered saline (P_i/NaCl, pH 7.2), and triplicate culture dishes were incubated at 24°C with 500 μl of P_i/NaCl containing 0.15 nM ^{125}I-Ang-II (100,000 cpm) and 0.8% heat-inactivated bovine serum albumin (BSA). In addition, triplicate cultures were also incubated at 24°C with the same reaction mixture but also containing a 10,000-fold excess of unlabeled Ang-II. Following the incubation, cultures were rinsed rapidly four times with a total of 10 ml of ice-cold P_i/NaCl containing 0.8% BSA. Cells were dissolved in 500 μl of 0.2 M NaOH and transferred to plastic tubes. Each plate was rinsed with 500 μl of distilled water, which was combined with the original sample. The radioactivity in each sample was determined in the Beckman model 5500 γ counter with a counting efficiency of 75% for ^{125}I. Specific binding of ^{125}I-Ang-II to glial cells was calculated as a mean of triplicate samples, and obtained by subtracting radioactivity bound in the presence of unlabeled Ang-II from the total radioactivity bound.

Effects of Catecholamines on ^{125}I-Ang-II Binding in Glial Cultures. The specific binding of ^{125}I-Ang-II to glial cultures treated with catecholamines was determined as detailed above. Cultures were incubated with 1 nM–10 μM norepinephrine (NE) or dopamine for 4 hr; NE and dopamine were prepared in P_i/NaCl (pH 7.4) containing 10 μM L-ascorbic acid, and control incubations were made using the same solvent.

Light Microscopic Autoradiography. Cultures were washed twice and incubated with 0.3 nM ^{125}I-Ang-II (200,000 cpm) and 0.8% BSA in 0.5 ml P_i/NaCl for 60 min at 24°C. Cultures were also incubated with a 10,000-fold excess unlabeled ^{125}I-Ang-II for nonspecific binding. Cultures were rinsed with ice-cold P_i/NaCl and fixed with 3.7% glutaraldehyde in P_i/NaCl for 30 min at 4°C. After fixation, cultures were washed with P_i/NaCl, dehydrated through graded ethanols (30–100%), and air dried. Culture dishes were coated with Ilford K5 nuclear emulsion and stored at 5°C in light tight boxes for 2–4 weeks. The emulsion was developed, and the cultures were examined with a phase-contrast microscope.

Effect of Ang-II on Phosphatidylinositol Hydrolysis in Glial Cultures. Phosphatidylinositol hydrolysis by glial cells in culture was measured by following the accumulation of [^{3}H]inositol phosphates from prelabeled glial cells in the presence of lithium (17, 18). Glial cells, grown in 35-mm tissue culture dishes were incubated with [^{3}H]inositol (final specific activity 13.1 Ci/mol; 1 Ci = 37 GBq) for 72 hr at 37°C. The plates were washed free of unincorporated [^{3}H]inositol with two washes of Krebs–Ringer bicarbonate buffer (KRB) that has been preoxygenated with 5% CO_2/95% O_2 to a final pH of 7.4. Cultures were then incubated with increasing concentrations of Ang-II in the absence and presence of 1 μM [sarcosine^1 (Sar^1),Ile^8]Ang-II in 0.45 ml of KRB containing 10 mM LiCl (substituted isotonically for NaCl). This was followed by incubation of cells for 30 min at 37°C. The reaction was stopped by the addition of 0.75 ml of ice-cold methanol, the cells were scraped, and the contents were transferred to a tube with an additional 0.7 ml of methanol. Inositol phosphates were isolated by ion-exchange chromatography (17, 18).

Effects of Ang-II on [^{3}H]NE Uptake in Glial Cultures. [^{3}H]NE (New England Nuclear) uptake in glial cultures was measured as detailed previously (13, 19). To determine the effects of Ang-II (1 μM) on [^{3}H]NE uptake, cultures were incubated with the peptide for 10 min before the NE-uptake experiment. In addition, Ang-II was included in the NE-uptake reaction mixture, and uptake was assayed for 5 min.

Materials. One-day-old rats of Wistar–Kyoto strain were obtained from our breeding stock that had originated from Charles River Breeding Laboratories. DMEM, P_i/NaCl, and fetal calf serum were purchased from GIBCO. Deoxyribonuclease and 1× crystalline trypsin (150 units/mg) were from Worthington. BSA fraction 5, cytosine arabinoside, poly-L-lysine, Ang-II, [Sar^1,Ala^8]Ang-II, and [Sar^1,Ile^8]Ang-II were obtained from Sigma. [^{3}H]Inositol (15.6 Ci/mmol) was obtained from Amersham. Ang-II was iodinated by the method of Düsterdieck and McElwee (20) with a resulting specific activity of 1500–1800 μCi/μg (7). The iodinated Ang-II migrated as a homogeneous peak on a reverse-phase HPLC system.

RESULTS

Kinetic Characterization of Ang-II Receptors in Glial Cultures. Glial cultures derived from the hypothalamus and brain stem regions of 1-day-old Wistar–Kyoto strain rats specifically bound ^{125}I-Ang-II. Binding of ^{125}I-Ang-II at 24°C increased rapidly with time and reached a plateau between 45 and 60 min (Fig. 2*A*). Nonspecific binding during the same time period remained low and did not exceed 5–7% of the total binding. After reaching equilibrium binding, the glial cultures were washed with ice-cold P_i/NaCl to remove unbound ^{125}I-Ang-II and were then incubated in P_i/NaCl to determine the rate of dissociation of bound radioactivity from the cells. The bound ^{125}I-Ang-II dissociated from the cells in a time-dependent manner reaching maximum dissociation of 90% in 100 min with a dissociation $t_{1/2}$ of 6–7 min (Fig. 2*B*), suggesting a rapid association and dissociation of Ang-II to glial cultures. The association and dissociation data from Fig. 2 were analyzed according to first-order kinetics (5, 8). When data from Fig. 2*A* were plotted as $\ln[B_{eq}]/([B_{eq}] - [B])$ against time, where $[B_{eq}]$ is the concentration of bound ^{125}I-Ang-II at equilibrium and [B] is the concentration of bound Ang-II at a given time point, straight lines were obtained, giving the apparent association constant $k_1 = 4 \times 10^6$ $M^{-}\cdot sec^{-1}$ (Fig. 3*A*). Data from Fig. 2*B* showing the dissociation of bound Ang-II were also analyzed according to the first-order kinetics, and a plot of $\ln([B]/[B_0])$ against time (where $[B_0]$ is the concentration of bound ^{125}I-Ang-II at 0 time, and [B] is the concentration of bound ^{125}I-Ang-II at a given time) gave a straight line (Fig. 3*B*). The apparent dissociation rate constant k_{-1} obtained from these data was $2.8 \times 10^{-4}\cdot sec^{-1}$. Comparison of the K_1 and K_{-1} values of the glial cultures with those of the neuronal cultures (8) indicated similar values for

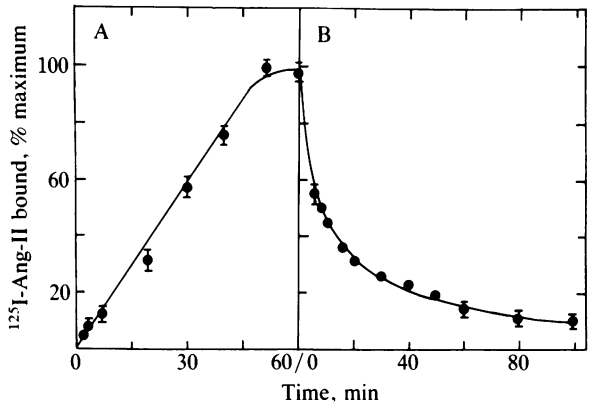

FIG. 2. Association and dissociation of ^{125}I-Ang-II binding to glial cultures. (*A*) Time course of ^{125}I-Ang-II binding to glial cultures from rat brain. Cultures, in triplicate, were rinsed with P_i/NaCl and incubated with 0.5 ml of P_i/NaCl containing ^{125}I-Ang-II (191,033 cpm) and 0.8% BSA in the absence and presence of unlabeled Ang-II for the indicated times. The cpm bound per culture at 60 min was 14,752 ± 886. Data are presented as % maximum ^{125}I-Ang-II specifically bound, where maximum binding was 1.3 ± 0.2 fmol per mg of protein. (*B*) Dissociation of bound ^{125}I-Ang-II from glial cultures. Glial cultures were incubated for 30 min at 24°C with ^{125}I-Ang-II (130,728 cpm) and were then washed to remove unbound radioactivity. Cultures, prelabeled with ^{125}I-Ang-II, were incubated with 2 ml of P_i/NaCl and 0.8% BSA at 24°C. At the indicated time periods, cultures were rinsed, cells were digested with 0.2 M NaOH, and radioactivity was determined. Data are presented as the mean of triplicate determination ± SEM and are represented as percent of control. The cpm for 30-min binding before the dissociation experiment were 6784 ± 121 per culture dish.

both cell types. Specificity of ^{125}I-Ang-II binding to glial cultures is shown in Fig. 4. Ang-II inhibited binding of ^{125}I-Ang-II in a dose-dependent fashion with an IC_{50} of 6.3 nM. In contrast, Ang-I and Ang-III were much less potent in competing for ^{125}I-Ang-II binding in these cells, with IC_{50} values of 500 nM and 630 nM, respectively. [Sar^1,Ile^8]Ang-II and [Sar^1,Ala^8]Ang-II, both antagonists of Ang-II receptors, competed for Ang-II binding in a dose-dependent manner. IC_{50} for [Sar^1,Ala^8]Ang-II was 1 nM and for [Sar^1,Ile^8]Ang-II was 31 nM. Structurally unrelated neuropeptides—neurotensin, insulin, and arginine vasopressin—did not compete for ^{125}I-Ang-II binding.

Specific binding of ^{125}I-Ang-II to glial cultures was saturable as a function of Ang-II concentrations. Scatchard analysis of saturation data revealed a straight line indicating the presence of a single population of binding sites (Fig. 5)

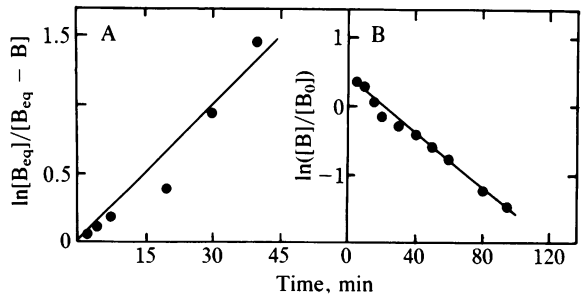

FIG. 3. Kinetic analysis of association and dissociation of ^{125}I-Ang-II to glial cultures. (*A*) Association data from Fig. 2*A* are plotted according to the first-order rate equation, and the association rate constant $\{\ln[B_{eq}]/([B_{eq}]-[B])\}$ was calculated as described (5, 8). (*B*) Kinetic analysis of the dissociation of ^{125}I-Ang-II from glial cultures. Dissociation data from Fig. 2*B* are plotted according to the first-order equation as described (5, 8), and the dissociation rate constants $\ln([B]/[B_0])$ were calculated.

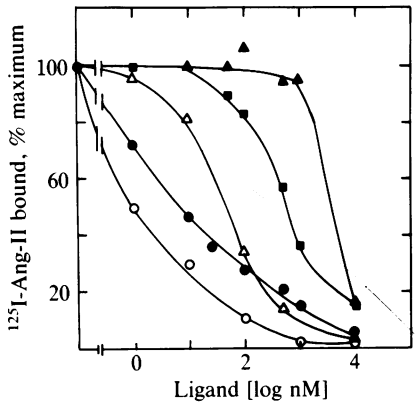

FIG. 4. Competition of binding of ^{125}I-Ang-II to glial cultures by various agonists and antagonists. Glial cultures were incubated with ^{125}I-Ang-II (112,150 cpm) containing the indicated concentrations of Ang-II (●), [Sar^1,Ala^8]Ang-II (○), [Sar^1,Ile^8]Ang-II (△), Ang-III (■), and Ang-I (▲) for 30 min at 24°C in the presence of ^{125}I-Ang-II, essentially as described. Each point represents the mean of triplicate determinations, and specific binding was 90% of the total ^{125}I-Ang-II bound and represented 4142 ± 43 cpm per culture dish.

with a dissociation constant (K_d) of 1.1 nM and a B_{max} of 110 fmol/mg of protein.

Autoradiography. Light-microscopic autoradiography of glial cultures was done to further confirm the presence of ^{125}I-Ang-II binding sites in these cultures. Fig. 6 shows that the cells containing silver grains representing ^{125}I-Ang-II were morphologically similar to the astrocytic glial type. Cultures incubated with ^{125}I-Ang-II in the presence of an excess of unlabeled Ang-II showed no significant silver grains on the cells (data not shown), supporting specificity of ^{125}I-Ang-II binding to these cells. These autoradiographic results further indicate that glial cells in culture, like neurons, contain Ang-II-specific binding sites.

Effects of Ang-II on Glial Cells. The effect of Ang-II on phosphatidylinositol hydrolysis was studied as a function of Ang-II concentration in glial cultures. Fig. 7 shows that Ang-II caused a dose-dependent stimulation of phosphatidylinositol formation. Significant stimulation was observed with concentrations as low as 0.1 nM Ang-II, and a maximum response of 2-fold was observed with 100 nM Ang-II. This stimulatory effect of Ang-II on phosphatidylinositol release was mediated by Ang-II-specific receptors because it was completely blocked by the Ang-II receptor antagonist [Sar^1,Ile^8]Ang-II. Although the glial cell Ang-II receptor

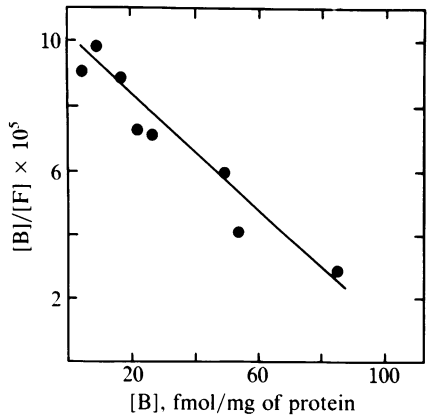

FIG. 5. Scatchard analysis of binding of ^{125}I-Ang-II to glial cultures. Glial cultures were incubated with increasing concentrations of ^{125}I-Ang-II (0.1–2 nM) for 30 min at 24°C. The saturation data were analyzed according to Scatchard to determine the K_d and the B_{max}.

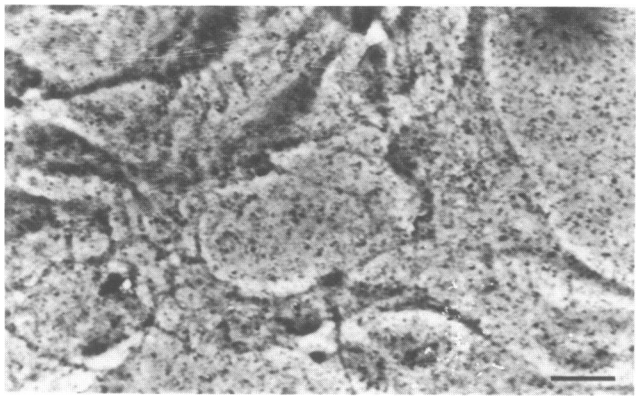

FIG. 6. Light-microscopic autoradiograph of ^{125}I-Ang-II binding to glial cultures. Glial cultures were processed for autoradiography as described, and clusters of cells containing silver grains are shown. Bar = 10 μM.

appears to be coupled to phosphatidylinositol hydrolysis, similar experiments in neuronal cultures indicated that the neuronal Ang-II receptors do not show a marked phosphatidylinositol response.

Our previous studies have shown that Ang-II has profound effects on catecholamine metabolism in neuronal cultures prepared from the hypothalamus and brain stem regions of 1-day-old rats (13, 14). The effect of Ang-II on [^{3}H]NE uptake in glial cultures was studied to determine the possible similarities in Ang-II effects on catecholamine metabolism between glial and neuronal cultures. Glial cultures incubated with 0.1 μM [^{3}H]NE at 37°C for 5 min in the absence or presence of Ang-II (1 μM). Ang-II did not significantly alter [^{3}H]NE uptake compared with control cells (2.05 ± 0.5 nmol/mg of protein/5 min [^{3}H]NE uptake in control versus 2.1 ± 0.7 nmol/mg of protein/5 min [^{3}H]NE uptake in Ang-II treated cells).

Effect of Catecholamine on Ang-II Receptors. Incubation of neuronal cultures from hypothalamus and brain stem with various concentrations of catecholamine downregulates Ang-II receptors, an effect mediated via α_1-adrenergic receptors (11). In the present study, identical incubations of glial cultures with 1 nM–10 μM NE or dopamine resulted in no significant changes in Ang-II-specific binding (see Table 1). If anything, Ang-II-specific binding increased with the lower concentrations of dopamine, though this effect was statistically insignificant.

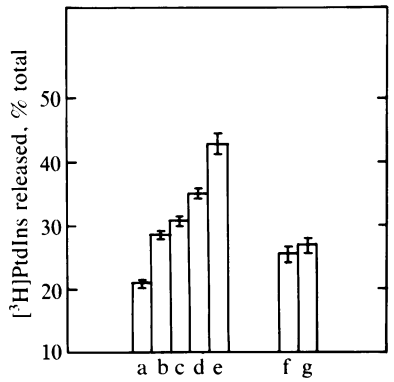

FIG. 7. The effect of Ang-II on formation of phosphatidylinositol (PtdIns) in glial cultures. Glial cultures were prepared and labeled with [^{3}H]inositol for 72 hr as described. They were then incubated either in the absence (a) or presence of Ang-II (b, 0.1; c, 1; d, 10; e, 100 nM) or in the presence of [Sar^1,Ile^8]Ang-II (1 μM) or of [Sar^1,Ile^8]Ang-II and Ang-II (f and g, 1 μM and 100 nM, respectively) for 10 min at 37°C in the presence of lithium.

Table 1. Effects of NE and dopamine on ^{125}I-Ang-II binding in glial cultures

Treatment	Conc., nM	^{125}I-Ang-II binding, fmol/mg of protein
None	0	15.2 ± 1.4
NE	1	17.9 ± 1.9
NE	100	16.3 ± 0.9
NE	1,000	16.5 ± 0.7
NE	10,000	15.5 ± 2.3
DA	1	18.4 ± 2.7
DA	100	13.7 ± 1.4
DA	1,000	15.2 ± 0.9
DA	10,000	15.6 ± 1.8

Glial cultures were incubated with the indicated concentrations of NE or dopamine at 37°C for 4 hr. Cultures were washed and used to quantitate ^{125}I-Ang-II-specific binding with the use of 0.40 nM ^{125}I-Ang-II essentially as described. Data are presented as means ± SEM; no significant differences between the groups were observed. This is a representative experiment that has been repeated three times with similar findings.

DISCUSSION

We have demonstrated that glial cells in primary culture from the hypothalamus and brain stem regions of 1-day-old normotensive rat contain specific Ang-II receptors and that these brain receptors on the glial cells appear to be functionally distinct from the Ang-II receptors on the neuronal cells in primary culture from the same brain regions.

Examination of glial cultures using phase-contrast microscopy revealed confluent monolayers that consisted of large, flat cells. The glial cultures contained 95–98% astrocytes, as evidenced by the use of antibodies to glial fibrillary acidic protein, with no significant presence of fibroblasts or oligodendrocytes. This is in contrast to neuronal cultures, which contain 80–85% neurons with the remainder of the cells being glia (8).

The binding of ^{125}I-Ang-II to glial cells in culture is specific; 90–95% of the label is displaced by an excess of unlabeled Ang-II. The binding is rapid and reversible, exhibiting similar association and dissociation constants to those found in neuronal cultures (8) and brain membranes prepared from a block of brain containing hypothalamus, thalamus, septum, and midbrain (5, 12). Saturation and Scatchard analyses of ^{125}I-Ang-II binding to glial cultures revealed a single population of high-affinity receptors. The B_{max} and K_d for these receptors were similar to the values observed in neuronal cultures and in brain membranes (5, 8, 12). Thus, the Ang-II receptors present in neuronal and glial cells appear identical from a kinetic standpoint. In contrast with kinetic properties, apparent differences exist between the glial and neuronal Ang-II receptors. Competition of ^{125}I-Ang-II binding to glial cultures with Ang-II and various analogues and antagonists revealed the following rank order of potency: Ang-II = [Sar^1,Ala^8]Ang-II > [Sar^1,Ile^8]Ang-II >> Ang-I > Ang-III. Similar experiments in neuronal cultures have revealed a different rank order of potency: Ang II >> [Sar^1,Ala^8]Ang-II >> Ang-I = Ang-III (8). These apparent differences in specificity of [Sar^1,Ala^8]Ang-II for Ang-II receptors between the two brain cell types may reflect functional differences between the receptors. Light-microscopic autoradiography revealed specific Ang-II receptors associated with astrocytic glia in the glial cultures. Together with our previous findings that neuronal cells in culture contain specific Ang-II receptors associated with both the cell bodies and neurites (8), the present findings suggest two separate populations of receptors—i.e., neuronal and glial.

The Ang-II receptors in glial cells appear to be functionally distinct from those in neuronal cultures. For example, this

study has demonstrated that Ang-II stimulates the hydrolysis of phosphatidylinositol, an effect mediated via specific Ang-II receptors in glial cultures. By contrast, in neuronal cultures Ang-II has little or no such receptor-mediated effects on phosphatidylinositol hydrolysis. In addition, we have previously shown that Ang-II stimulates NE uptake into neuronal cultures, an effect mediated via Ang-II receptors (13). This effect is absent in glial cultures, as demonstrated in the present study. Finally, the regulation of neuronal and glial Ang-II receptors also appears to be different. The down-regulatory effects of NE, which are mediated via α_1-adrenergic receptors in neuronal cultures (11), are not seen in glial cultures. Though two of the functional distinctions between neurons and glia are based on negative findings, we believe them to be true differences for the following reasons. (*i*) The lack of effect of NE on Ang-II-receptor regulation in glia and the inability of Ang-II to increase [^{3}H]NE uptake into glia are not due to different experimental conditions compared with the prior neuronal experiments; the experimental conditions and protocols used were identical to those in the neuronal experiments. (*ii*) Other studies have clearly shown the presence of α_1-adrenergic receptors. (*iii*) Lastly, astrocytic glia contain both sodium-independent (13) and sodium-dependent (21) NE uptake sites, thus demonstrating that glia have the ability to transport NE.

Thus, our studies show the presence of two distinct populations of Ang-II receptors in the central nervous system—one population associated with neurons that mediates profound influence on catecholamine metabolism, possesses distinct specificity, and is regulated by catecholamine receptors, and the other population associated with glial cells, which is coupled to phosphatidylinositol hydrolysis, are neither regulated by catecholamine receptors nor influenced by catecholamine metabolism. These separate populations can be visualized using light-microscopic autoradiography.

The physiological significance of this receptor on neuronal and glial cells is not fully understood. However, from this and previous studies (8, 11, 14, 15), it is tempting to speculate that the neuronal receptor has an important role in the neuromodulatory effects of Ang-II, especially in relation to interactions with catecholamines, whereas the glial receptor may play a role in the feedback control of the renin–Ang-II system recently demonstrated in these cells. The suggested importance of the central nervous Ang system in blood pressure regulation (22) and water balance (23) further encourages speculation that glial Ang-II receptors have a role in these physiological functions.

The authors thank Cathy Morse for her expert technical assistance and Elizabeth Albert and Sue Rueth for preparation of cell cultures. This work is supported by National Institutes of Health Grants HL33610 and NS 19441. M.K.R. is an Established Investigator of the American Heart Association.

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