Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 2914–2918, March 1995
Neurobiology

Delivery of angiotensin II type 1 receptor antisense inhibits angiotensin action in neurons from hypertensive rat brain

(gene transfer/spontaneously hypertensive rat/norepinephrine transporter/gene expression)

DI LU AND MOHAN K. RAIZADA*

Department of Physiology, University of Florida, College of Medicine, Gainesville, FL 32610

Communicated by George K. Davis, University of Florida, Gainesville, FL, December 12, 1994

ABSTRACT Increased brain angiotensin II (AII) type 1 receptor (AT_1R) expression has been implicated in the hyperactive brain angiotensin system and the development and maintenance of hypertension in the genetically spontaneously hypertensive (SH) rat. Neuronal cells in primary culture from the cardioregulatory-relevant brain areas (hypothalamus/brainstem) mimic increased brain AT_1R gene expression and AT_1R function of the adult SH rat. They have been utilized in the present study to determine whether cellular actions of AII could be regulated by the transfer of AT_1R antisense (AT_1R-AS) with the use of a retroviral-mediated gene delivery system developed for the central nervous system cultures. AII stimulates norepinephrine (NE) uptake in neuronal cultures of both normotensive (Wistar Kyoto) and SH rat brains. This neuromodulatory action is mediated by the AT_1R subtype, is significantly higher in SH neurons, and is associated with a parallel stimulation of mRNAs for c-fos and NE transporter. Infection of neuronal cultures with a retrovirus vector that contains AT_1R-AS (LNSV-AT_1R-AS) results in an inhibition of AT_1R-mediated stimulation of both c-fos and NE transporter mRNA, as well as NE uptake in both strains of rats; however, the inhibition is more pronounced in SH neurons compared with Wistar Kyoto rat brain neurons. The higher sensitivity of the SH rat brain neurons is further supported by our observation that a certain dose of LNSV-AT_1R-AS that fails to induce inhibition of cellular actions of AII in WKY neurons causes a significant inhibition of AII actions in SH neurons. These observations show that retrovirally mediated delivery of AT_1R-AS could be used to selectively control the actions of AII in primary neuronal cultures from SH rat brain.

Angiotensin II (AII), a potent vasoconstrictor hormone, elicits profound physiological actions mediated by the brain, such as increased blood pressure (BP), sympathetic out-flow, and altered baroreceptor function (1, 2). These actions of AII are mediated by activation of the AII type 1 receptor (AT_1R) subtype in cardioregulatory-relevant areas of the brain (1, 2). The physiological importance of the brain AII system in control of BP is further heightened by observations that the spontaneously hypertensive (SH) rat, a genetic model for essential hypertension, exhibits hyperactivity of the brain AII system, including AII receptors and the mRNA for AT_1R (2). Additionally, intervention in the activity of this system with the use of converting enzyme inhibitors, AII, or AT_1R antagonists decreases high BP and normalizes other pathophysiological changes associated with the hypertensive state in SH rat (2). These and other observations (4) point out that control of AT_1R gene can be an important step in the regulation of AII-mediated brain actions on BP and, thus, control of hypertension in the SH rat.

Our laboratory has developed an *in vitro* neuronal cell culture model from the hypothalamus/brainstem areas of 1-day-old Wistar Kyoto (WKY) and SH rat brains in an attempt to investigate the cellular and molecular basis of the hyperactive brain AII system in SH rats (2). These studies have established that neuronal cultures from SH rats express increased AII, AII receptors, and AII stimulation of norepinephrine (NE) uptake system, changes similar to those observed in the brain of adult SH rats (2–4). In addition, recent experiments have shown that AT_1R gene expression and AT_1R activity are also increased in neuronal cultures of the SH rat brain (5). This increase is associated with a parallel increase in the mRNAs for both $AT_{1A}R$ and $AT_{1B}R$ subtypes (5). These observations have led us to conclude that increased AT_1R gene expression in the SH rat brain may be genetically linked with development of the hypertensive state in this animal model. Thus, neuronal cultures provide us with an excellent *in vitro* investigative tool to study the mechanism of increased AT_1R gene expression and to manipulate the expression of this gene in an attempt to control AII-mediated cellular actions in WKY and SH rat brains. In the present study, we have constructed a retroviral vector containing AT_1R anti-cDNA to deliver AT_1R antisense (AT_1R-AS) into neuronal cultures. Our studies show that the delivery of AT_1R-AS results in attenuation of AT_1R-mediated cellular actions in WKY and SH rat brain neuronal cultures.

MATERIALS AND METHODS

Construction of Retroviral AT_1R-AS (LNSV-AT_1R-AS) and Preparation of Medium Containing LNSV-AT_1R-AS Viral Particles. A retroviral vector, LNSV, was used to deliver $AT_{1B}R$-AS into neurons because of its success in delivering genes to cells of neural origin (6, 7). The $AT_{1B}R$-AS (nt −132 to +1128) was cloned in the LNSV vector and transfected to a packaging cell line, PA317. After selection by G418, the medium containing viral particles that expressed $AT_{1B}R$-AS was collected and used for infection essentially as described (8).

Preparation and Infection of Neuronal Cultures with LNSV-$AT_{1B}R$-AS. Hypothalamus/brainstem areas of 1-day-old WKY and SH rat brains were dissected and brain cells were dissociated by trypsin. The hypothalamic block contained the paraventricular nucleus, the supraoptic, anterior, lateral, posterior, dorsomedial, and ventromedial nuclei. The brainstem block contained medulla oblongata and pons. Dissociated cells were plated in poly-(L-lysine)-precoated tissue culture dishes or 8-well slides (Nunc) at 800 cells per mm^2 and grown for 8 days essentially as described (5, 9, 10). Cultures were incubated with viral particles [4×10^5 colony-forming units (cfu)/ml]

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AII, angiotensin II; AT_1R, AT_2R, etc., AII type 1 receptor, AII type 2 receptor, etc.; SH, spontaneously hypertensive; AT_1R-AS, AT_1R antisense; NE, norepinephrine; WKY, Wistar Kyoto; NET, NE transporter; BP, blood pressure; cfu, colony-forming unit(s); RT, reverse transcriptase.

*To whom reprint requests should be addressed at: Department of Physiology, University of Florida, Box 100274, Gainesville, FL 32610.

containing LNSV-$AT_{1B}R$-AS for 48 hr at 37°C. In initial experiments, we have determined that these culture conditions are optimum for infection and expression of $AT_{1B}R$-AS by neuronal cultures.

Measurements of c-fos mRNA and NE Transporter (NET) mRNA. Neuronal cultures were established in 35-mm tissue culture dishes. They were incubated with 100 nM AII for 20 min (c-fos) and 4 hr (NET). Poly(A)$^+$ RNA was isolated by using Dynal beads (Dynal, Great Neck, NY) and mRNAs for c-fos and NET were determined by a quantitative reverse transcriptase (RT)–PCR method, the validity of which has been established (11). The specific primers used were based on published sequences (12–14) and were as follows: c-fos, sense (5′-AGGAGGGAGCTGACAGATA-3′) and antisense (5′-CCTGGCTCACATGCTACTA-3′); NET, sense (5′-CCG-CATCCATGCTTCTGGCGCGGATGAA-3′) and antisense (5′-GGGCAGGCTCAGATGGCCAGCCCAGTGTT-3′); β-actin, sense (5′-GAGAAGATGACCCAGATCATGT-3′) and antisense (5′-ACTCCATGCCCAGGAAGGAAG-3′).

RT–*in Situ* PCR Detection of Native AT_1R and $AT_{1B}R$-AS in Neuronal Cultures. Cultures established in 8-well Nunc slides were fixed with 10% (vol/vol) buffered formalin (pH 7.4) for 18 hr at 4°C and treated with trypsin (10,000 N^{α}-benzoyl-L-arginine ethyl ester (BAEE) units/mg) for 45 min at room temperature followed by RNase-free DNase treatment at 37°C (10 units/μl) overnight. After two rinses with diethyl pyrocarbonate H_2O, dehydration, and air drying, RT reaction solution (10 μl) was added, which contained 5 mM $MgCl_2$, 1 × RT buffer (50 mM Tris·HCl, pH 8.3/75 mM KCl/3 mM $MgCl_2$/10 mM *dl*-dithiothreitol), all four dNTPs (each at 1 mM), 5 units of RNase inhibitor, 1 μM $AT_{1B}R$-AS primer (5′-CCAGAAAGCCGTAGAACAGAGGG-3′), and 1 unit of avian myeloblastosis virus RT, and the RT reaction was done essentially as described (8). This was followed by 18 cycles of PCR with the use of $AT_{1B}R$ primers (antisense, 5′-CCAGAAAGCCGTAGAACAGAGGG-3′; sense, 5′-CTT-TCTTCTCAATCTCGCCTTGG-3′) and digoxigenin-11-dUTP (15). After PCR, coverslips were removed and cells were used for immunodetection of digoxigenin-dUTP incorporated as described (8). An identical protocol was also used to detect $AT_{1B}R$-AS in neuronal cultures except $AT_{1B}R$ sense primer was substituted for antisense primer in the RT reaction.

RESULTS

Neuronal cultures from hypothalamus/brainstem of SH rat brain express 2- to 4-fold higher levels of functional AT_1R compared with the neuronal cultures of WKY rat brain (2, 5). In view of the observation that these cultures contain 10% astroglial cells, the first objective in our study was to determine the localization of these AT_1R and compare the number of brain cells expressing these receptors in both WKY and SH rat neuronal cultures. Fig. 1 shows an RT–*in situ* PCR detection of AT_1R transcript in both WKY and SH neuronal cultures. Quantitative analysis revealed that 30 ± 5% (n = 3; total number of cells counted was 1×10^5) of cells of neuronal morphology from either WKY or SH rat brain cultures stain positive. Thus, the increase in the AT_1R gene expression observed previously (5) is a result of an increase in the number of AT_1Rs per cell in SH rat brain neuronal cultures.

A retroviral vector (LNSV) containing $AT_{1B}R$-AS was constructed and viral particles were used to infect neuronal cultures. Fig. 2 shows a representative RT–*in situ* PCR experiment demonstrating the expression of $AT_{1B}R$-AS in both WKY and SH rat brain neurons. The transcript is localized in the perinuclear region of the neuronal cell soma, indicating a successful integration and transcription of $AT_{1B}R$-AS neuronal culture of both strains of rats.

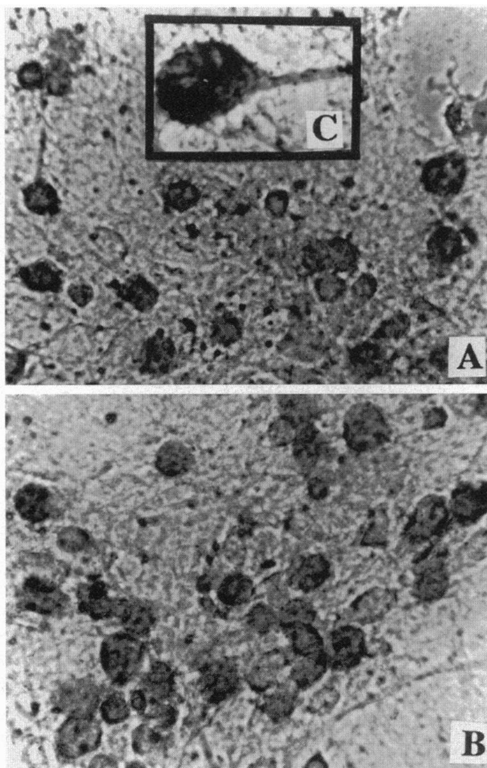

FIG. 1. RT–*in situ* PCR detection of native AT_1R transcript in neuronal cultures of WKY and SH rat brains. (*A*) WKY rat brain neuronal culture. (*B*) SH rat brain neuronal culture. (×1000.) (*C*) Single neuron. (×2500.) Control cultures, where RT was not included, were also analyzed in parallel and showed no staining.

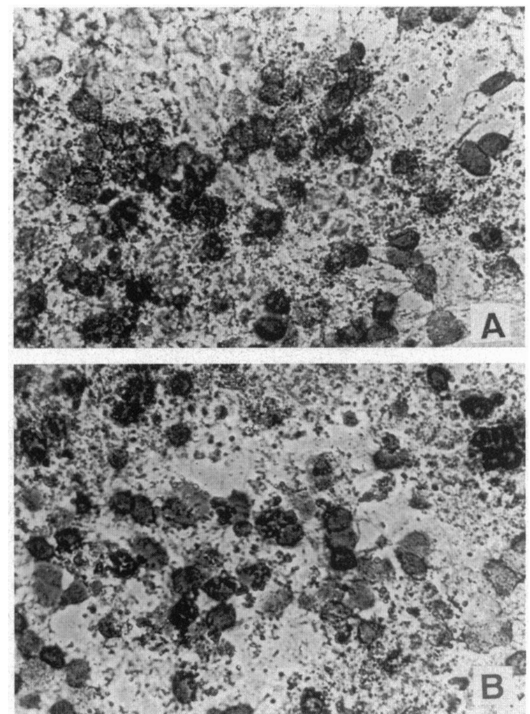

FIG. 2. RT–*in situ* PCR detection of $AT_{1B}R$-AS in neuronal cultures of WKY and SH rat brains. Neuronal cultures from the hypothalamus/brainstem areas of 1-day-old WKY (*A*) and SH (*B*) rat brains were grown for 8 days, incubated with viral particles (4×10^5 cfu per 10^3 cells) containing LNSV-AT_1R-AS for 48 hr at 37°C, and subjected to RT–*in situ* PCR essentially as described (8). Cultures not infected with LNSV-AT_1R-AS or infected with LNSV-β-gal did not show any staining after RT–*in situ* PCR for AT_1R-AS. (×1000.)

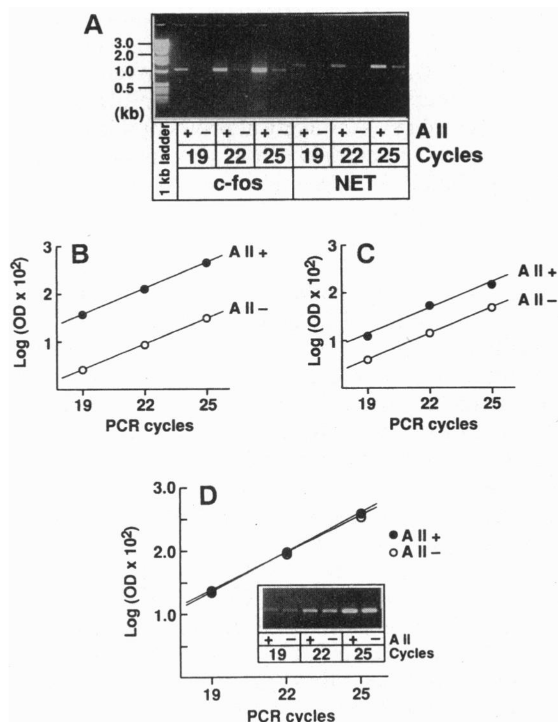

FIG. 3. Quantitation of AII-stimulated c-fos and NET mRNA levels by RT–PCR. Neuronal cultures were established in 35-mm tissue culture dishes. They were incubated with 100 nM AII for 20 min (c-fos) and 4 hr (NET). mRNA levels were determined by a quantitative RT–PCR (11) with the use of c-fos, NET, and actin specific primers. (*A*) RT–PCR products for c-fos and NET at 19, 22, and 25 cycles. (*B* and *C*) Densitometric quantitation of AII-stimulated c-fos (*B*) and NET (*C*) mRNAs at various PCR cycles. Data are the logarithm of the mean OD values from three experiments. (*D*) Densitometric quantification of β-actin mRNA at various PCR cycles. Data are the logarithm of the mean OD values from three experiments. (*Inset*) Representative products of β-actin RT–PCR.

With the evidence that $AT_{1B}R$-AS could be expressed, our next objective was to determine whether cellular actions of AII are influenced by this expression. The ability of AII to stimulate c-fos, NET genes, and [^{3}H]NE uptake was utilized as examples of AII's cellular actions. The rationale for choosing these responses is based on our previous studies (2) that showed that AII-mediated neurotransmission is associated with the stimulation of NET system and may involve c-fos. Fig. 3 shows that AII stimulates mRNAs for c-fos and NET as measured by RT–PCR. Validity of the quantitative nature of this protocol has been established (11). AII causes a 15- and 3-fold stimulation of c-fos and NET mRNAs, respectively. The stimulation is logarithmically linear within 19–25 cycles of PCR. AII has no effect on actin mRNA, and thus, all our PCR data have been normalized for actin. Fig. 4 shows comparison of AII stimulation of NET mRNA in neuronal cultures of WKY and SH rat brains. AII causes a time-dependent stimulation in both strains of neurons with a maximal effect in 4–24 hr. Although the time course of the AII effect is comparable, the fold stimulation is significantly higher in SH rat brain neurons compared with WKY neurons (2.6 ± 0.2 times vs. 4.9 ± 0.2 times, respectively, at 24 hr). Fig. 4*B* shows the effects of AII-receptor subtype antagonists on stimulation of NET mRNA. AII stimulation is completely blocked by losartan but not by PD123319, indicating that the effect is mediated by AT_1R subtype in both strains of neuronal cultures.

Next, we studied the effects of LNSV-$AT_{1B}R$-AS infection of WKY and SH rat brain neurons on AII stimulation of c-fos and NET mRNA levels. Fig. 5 shows that AII stimulation of c-fos mRNA is significantly attenuated in LNSV-$AT_{1B}R$-AS-

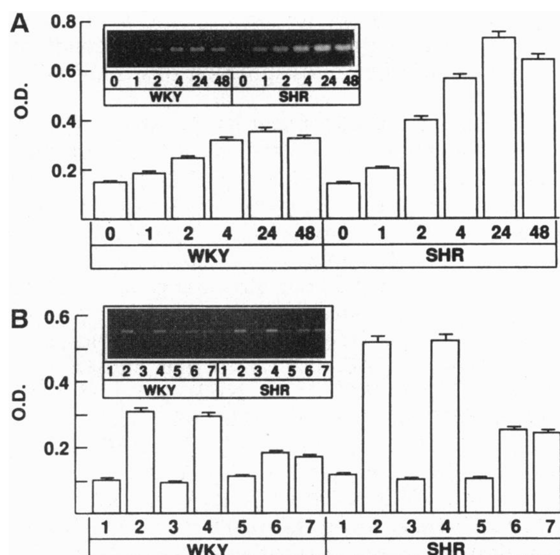

FIG. 4. Effect of AII on NET mRNA in neuronal cultures of WKY and SH rat brains. (*A*) Time course. Eight-day-old neuronal cultures of WKY and SH rat brains were incubated with 100 nM AII for various lengths of time (in hours), as indicated under bars and lanes. NET mRNA was quantitated. Data are the mean ± SEM ($n = 3$) and are normalized for β-actin. (*Inset*) A representative picture of the agarose gel. Zero time vs. 2- to 48-hr AII treatment showed a significant difference ($P < 0.01$) for both WKY and SH cultures. WKY vs. SH rat neurons showed a significant difference in AII-stimulated NET ($P < 0.01$). (*B*) Effect of antagonists. Neuronal cultures of WKY and SH rat brains were incubated without (Exp. 1, 3, and 5) or with (Exp. 2, 4, 6, and 7) 100 nM AII. Cultures also contained 1 μM PD123319 (Exp. 3, 4, and 7), 1 μM losartan (Exp. 5–7), or both (Exp. 7) for 4 hr at 37°C. Data are the mean ± SEM ($n = 3$) and are normalized for β-actin. (*Inset*) A representative picture of the agarose gel. The stimulation by AII (Exp. 1 vs. 2) and its inhibition by losartan (Exp. 2 vs. 6) were significant ($P < 0.01$).

infected neurons in both strains of rats. However, comparison of this inhibitory action indicates that it is 40% greater in SH rat brain neurons than in WKY neurons. The expression of $AT_{1B}R$-AS has no effect on basal c-fos mRNA. Fig. 6 shows a similar experiment where NET mRNA levels are measured. As observed for c-fos mRNA, AII stimulation of NET mRNA is higher in SH rat neurons compared with WKY control (5.6-fold vs. 3.0-fold, respectively). Infection with LNSV-AT_1R-AS

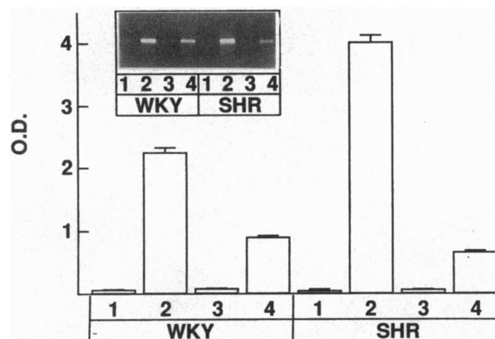

FIG. 5. Stimulation of c-fos mRNA by AII in uninfected and LNSV-AT_1R-AS-infected WKY and SH rat brain neurons. Neuronal cultures of WKY and SH rat brains were infected (Exp. 3 and 4) with LNSV-AT_1R-AS as described in Fig. 2. Uninfected (Exp. 1 and 2) cultures were used as controls. They were incubated without (Exp. 1 and 3) or with (Exp. 2 and 4) 100 nM AII for 20 min at 37°C and subjected to quantitative RT–PCR for c-fos as described in Fig. 3. Data are the mean ± SEM ($n = 3$). (*Inset*) A representative picture of the experiment is shown. Significant differences were noted when Exp. 1 was compared with Exp. 2 and when Exp. 2 was compared with Exp. 4 by a paired t test ($P < 0.01$).

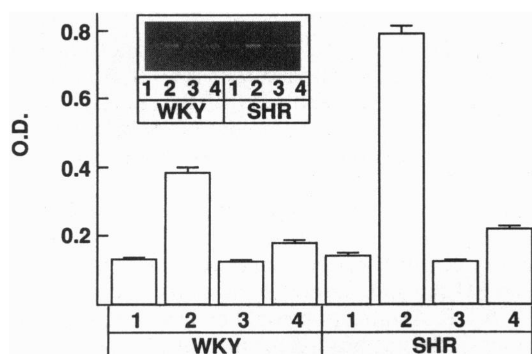

FIG. 6. Stimulation of NET mRNA by AII in uninfected and LNSV-AT_1R-AS-infected WKY and SH rat brain neurons. Neuronal cultures of WKY and SH rat brains were either infected (Exp. 3 and 4) or left uninfected (Exp. 1 and 2) and were incubated without (Exp. 1 and 3) or with (Exp. 2 and 4) 100 nM AII for 4 hr at 37°C. Data are the mean ± SEM (n = 3). (*Inset*) A picture of a representative experiment. Significant differences were found by t test when Exp. 1 vs. Exp. 2 and Exp. 2 vs. Exp. 4 were compared ($P < 0.01$).

attenuates this response and the level of this attenuation is 50% in WKY and 75% in SH rat brain neurons. This indicates that AII stimulation is twice as sensitive to LNSV-$AT_{1B}R$-AS expression in SH neurons compared with WKY neurons. This inhibitory action of LNSV-AT_1R-AS on AT_1R function was specific since AT_2R expression did not change. Fig. 7 shows a comparison of AII-stimulated [^{3}H]NE uptake in uninfected and LNSV-$AT_{1B}R$-AS-infected WKY and SH rat brain neuronal cultures. AII causes a 3-fold and 5.5-fold stimulation of specific [^{3}H]NE uptake in WKY and SH rat brain neuronal cultures, respectively. LNSV-$AT_{1B}R$-AS-infected cultures show reduced [^{3}H]NE uptake in response to AII. An inhibition of 34 and 55% is observed in WKY and SH neurons, respectively.

Finally, we determined the possibility of a preferential inhibition of AII's effects in SH rat brain neurons as a result of infection with controlled doses of LNSV-$AT_{1B}R$-AS. The rationale for this is based on the fact that the selective inhibition of the brain AII system by antagonists would decrease BP in SH rat only (2). Neuronal cultures were first

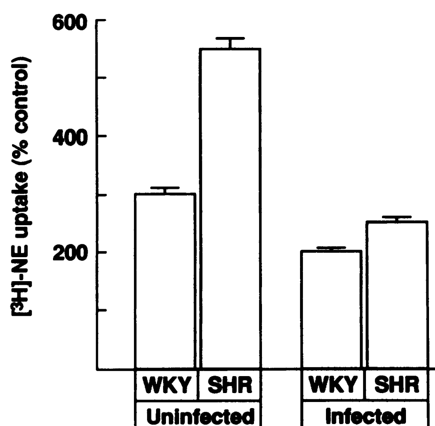

FIG. 7. Effect of AII on [^{3}H]NE uptake in uninfected and LNSV-AT_1R-AS-infected WKY and SH rat brain neurons. Neuronal cultures of WKY and SH rat brains were established in 35-mm dishes, infected with LNSV-AT_1R-AS as described in Fig. 2. Uninfected cultures were used as controls. They were incubated with 100 nM AII for 4 hr, and [^{3}H]NE uptake was quantitated essentially as described (16). Data are the mean ± SEM (n = 3) and are percent increase by AII over control. The control represents maprotoline-sensitive [^{3}H]NE uptake in the absence of AII and was 0.25 pmol/mg of protein. Data are the mean ± SEM (n = 3). Significant differences ($P < 0.01$) were observed between uninfected WKY and SH rat neuronal cultures.

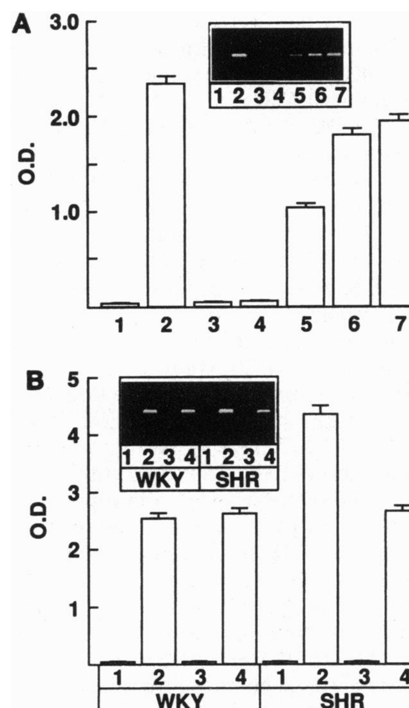

FIG. 8. Viral dose-dependent effects on AII-stimulated c-fos mRNA levels in WKY and SH rat brain neurons. (*A*) WKY neuronal cultures were incubated without (Exp. 1 and 2) or with the following amounts of LNSV-AT_1R-AS: 4×10^5 cfu per 10^3 cells (Exp. 3 and 4), 8×10^4 cfu per 10^3 cells (Exp. 5), 1.6×10^4 cfu per 10^3 cells (Exp. 6), and 3.2×10^3 cfu per 10^3 cells (Exp. 7) for 4 hr at 37°C essentially as described in Fig. 3. After infection, cultures were incubated without (Exp. 1 and 3) or with (Exp. 2 and 4–7) 100 nM AII for 20 min at 37°C and c-fos mRNA was measured. Data are the mean ± SEM (n = 3). (*Inset*) A representative picture of the experiment. (*B*) Uninfected (Exp. 1 and 2) and LNSV-AT_1R-AS-infected (Exp. 3 and 4) WKY and SH rat brain neuronal cultures (5×10^3 cells) were incubated without (Exp. 1 and 3) or with (Exp. 2 and 4) 100 nM AII for 20 min at 37°C for analysis of c-fos mRNA. Data are the mean ± SEM (n = 3). (*Inset*) A representative picture of the experiment.

infected with various doses of LNSV-AT_1R-AS (4×10^5 to 8×10^2 cfu per 10^3 cells) to determine a dose that fails to influence AII stimulation of c-fos mRNA in WKY rat brain. Fig. 8 shows that 5×10^3 cfu of LNSV-AT_1R-AS per 10^3 cells does not inhibit actions of AII stimulation of c-fos mRNA in WKY rat brain neurons. Neuronal cultures of SH rat brain were prepared, grown, and infected with 5×10^3 cfu of LNSV-AT_1R-AS per 10^3 cells in parallel with WKY cultures. Analysis of the data showed (Fig. 8) that infection causes a 40% inhibition of AII stimulation of c-fos mRNA in SH rat brain neurons but has no effect on WKY neurons.

DISCUSSION

Observations from this study establish that (*i*) retroviral vector can be used to deliver a foreign gene into neuronal cells in primary culture from rat brain, (*ii*) AT_1R stimulates c-fos and NET mRNA, a suggested cellular equivalent of neuromodulatory actions of AII *in vivo*, (*iii*) AT_1R-AS delivery attenuates AII-mediated actions in both WKY and SH rat brain neurons, and (*iv*) a selective attenuation of AII action could be demonstrated in the SH rat brain neurons with the use of an appropriate viral dose.

RT–*in situ* PCR data indicate that the AT_1R transcript is present in cells consistent with neuronal morphology. Further support of neuronal localization of AT_1R is derived from our many other observations: neuronal cells express a high-affinity Na^+-dependent NET whose activity is stimulated by the

interaction of AII with the AT_1R subtype (2, 4). In addition, AII fails to stimulate NET in cultures where neurons have been destroyed by KCl. Finally, astroglial cells prepared from the same brain areas of WKY and SH rats neither express differences in the numbers of AT_1Rs or the AT_1R mRNAs nor show any AII stimulation of NE uptake (2). Quantitative analysis revealed that 30% of neurons from both WKY and SH rat brain neuronal cultures stained positive for the AT_1R transcript. This indicated that an increase in the mRNA and B_{max} of AT_1R in the SH rat brain culture is a result of an increase in the AT_1R gene transcription, a conclusion further supported by the "nuclear run-on" assay (2, 17).

Central injection of AII activates catecholaminergic pathways that are associated with stimulation of sympathetic activity (18, 19). This neuromodulatory action of AII is, in part, responsible for the hypertensive action of AII and is elevated in the SH rat (1, 2, 18, 19). At the cellular level, the neuromodulation is shown to be associated with AII regulation of uptake, release, and synthesis of NE and stimulation of NET mRNA in neuronal cultures (2). Thus, we studied the effect of AT_1R-AS delivery on AII stimulation of NET system in neurons of WKY and SH rat brains. The data show that infection with LNSV-AT_1R-AS results in a significant attenuation of AII stimulation of c-fos mRNA, NET mRNA, and [^{3}H]NE uptake in both WKY and SH rat brain neurons. Although inhibition was seen in neuronal cultures of both strains of rat, the fold attenuation was significantly higher in SH neurons. This indicates that a higher sensitivity of AT_1R function in SH neurons compared with WKY neurons and is consistent with *in vivo* observations where intervention in AII-receptor function decreases BP in the SH rat (2). These observations raise the following important questions.

(*i*) Are these changes specific for AII? The following results support the notion that they are AII-specific: Stimulation of α_1-adrenergic receptors by NE increases c-fos mRNA in neuronal cultures. No significant difference in NE stimulation of c-fos mRNA is seen between uninfected and LNSV-AT_1R-AS-infected neuronal cultures. The mRNA for AT_2R (20) is also not altered in LNSV-AT_1R-AS-infected neurons compared with uninfected neurons. Additionally, basal levels of mRNA for other genes such as NET and β-actin are comparable between the uninfected and infected neuronal cultures. Infection of cultures with a similar construct of LNSV containing β-galactosidase neither changes AT_1R nor affects AII-mediated responses.

(*ii*) Does attenuation of AII stimulation of NET mRNA reflect a decrease in AII stimulation of NE uptake? Data shown in Fig. 7 indicate that, similar to NET mRNA, AII stimulation of [^{3}H]NE uptake was 83% higher and its attenuation after LNSV-AT_1R-AS infection was 60% greater in SH neurons compared with WKY neurons. This suggests the expression of AT_1R influences NE neurotransmission at both transcriptional and translational levels and confirms our contention that the expression of AT_1R is a rate-limiting step in the control of cellular actions of AII in these cultures.

(*iii*) Could AT_1R-mediated cellular actions be selectively inhibited in LNSV-AT_1R-AS-infected SH rat brain neurons? It is important to examine this in view of the fact that central injection of AII-receptor antagonists, in low doses, causes a decrease in BP only in SH rat and not in normotensive rat (2). The data in Fig. 8 show that a dose of LNSV-AT_1R-AS that fails to influence AII action in WKY neurons causes a 40% inhibition in SH neurons. This is a preliminary indication that this technique has the potential for its selective inhibition of AII action in SH rat brain.

Previous studies have shown that both brain and neuronal cultures express both $AT_{1A}R$ and $AT_{1B}R$ subtypes in relatively equal quantities (3, 5). Experiments presented in this study have been performed with the use of $AT_{1B}R$-AS and show that although the $AT_{1A}R$ and $AT_{1B}R$ subtypes are products of two distinct genes (21), the expression of $AT_{1B}R$-AS in neuronal cultures influences both subtypes of AT_1R. This would be consistent with the reported homology of 95–98% of the amino acid and nucleotide sequences between the two receptor subtypes (21).

In summary, this study points out that intervention in the synthesis and expression of AT_1R results in a significant inhibition of cellular and molecular responses of AII in both WKY and SH rat brain neurons and that selective conditions of LNSV-AT_1R-AS infection could be developed to influence AII actions in SH rat brain neurons only. Finally, these data are potentially significant because they indicate that cellular, molecular, and physiological actions of AII could be manipulated at the genetic level. The ability to conduct such experiments *in vitro* and *in vivo* not only should be important for elucidating the role of AT_1R and AT_2R subtypes in the physiology of AII actions but also should be significant for developing strategies toward a long-term control of AII-dependent hypertension.

We thank Ms. Elizabeth Brown for cell culture and Ms. Jennifer Brock for preparation and typing of this manuscript. D.L. is a postdoctoral fellow of the American Heart Association, Florida Affiliate. This work was supported by the National Institutes of Health Grant HL33610.

1. Saavedra, J. M. (1992) *Endocr. Rev.* **13,** 329–380.
2. Raizada, M. K., Lu, D. & Sumners, C. (1994) *Current Concepts: Tissue Renin-angiotensin Systems as Local Regulators in Reproductive and Endocrine Organs* (Plenum, New York), in press.
3. Raizada, M. K., Sumners, C. & Lu, D. (1993) *J. Neurochem.* **60,** 1949–1952.
4. Sumners, C. & Raizada, M. K. (1993) *Cellular and Molecular Biology of the Renin-Angiotensin System* (CRC, Boca Raton, FL).
5. Raizada, M. K., Lu, D., Tang, W., Kurian, P. & Sumners, C. (1993) *Endocrinology* **132,** 1715–1722.
6. Owens, G. C. & Boyd, C. J. (1991) *Development (Cambridge, U.K.)* **112,** 639–649.
7. Verity, A. N., Bredesen, D., Vonderscher, C., Handley, V. W. & Campagnoni, A. T. (1993) *J. Neurochem.* **60,** 577–587.
8. Lu, D., Yu, K. & Raizada, M. K. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1162–1166.
9. Raizada, M. K., Muther, T. F. & Sumners, C. (1984) *Am. J. Physiol.* **247,** C364–C372.
10. Sumners, C., Tang, W., Zelezna, B. & Raizada, M. K. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7567–7571.
11. Wang, A. M., Doyle, M. V. & Mark, D. F. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9717–9721.
12. Curran, T., Peters, G., Van Beveren, C., Teich, N. M. & Verna, I. M. (1982) *J. Virol.* **44,** 674–682.
13. Pacholczyk, T., Blakely, R. D. & Amara, S. G. (1991) *Nature (London)* **350,** 350–354.
14. Nudel, U., Zakut, R., Shani, M., Neuman, S., Levy, Z. & Yaffe, D. (1983) *Nucleic Acids Res.* **11,** 1759–1764.
15. Nuovo, G.-J., Forde, A., MacConnel, P. & Fahrenwald, R. (1993) *Am. J. Pathol.* **143,** 40–46.
16. Sumners, C., Muther, T. H. & Raizada, M. K. (1985) *Am. J. Physiol.* **248,** C488–C497.
17. Lu, D., Sumners, C. & Raizada, M. K. (1994) *J. Neurochem.* **62,** 2079–2084.
18. Steckelings, V., Lebrun, C., Quadri, F., Veltman, A. & Unger, T. (1992) *J. Cardiovasc. Pharmacol.* **19,** Suppl. 6, S73–S79.
19. Timmermans, P. B. M. W. M., Wong, P. C., Chiu, A. T., Herblin, W. F., Benfield, P., Carini, D. J., Lee, R. J., Wexler, R. R., Saye, A. M. & Smith, R. D. (1993) *Pharmacol. Rev.* **45,** 205–251.
20. Mukoyama, M., Nakajima, M., Horiuchi, M., Sagamura, H., Pratt, R. E. & Dzau, V. J. (1993) *J. Biol. Chem.* **268,** 24539–24542.
21. Inagami, T., Iwai, N., Sasaki, K., Yamano, Y., Bardhan, S., Chaki, S., Guo, D.-F. & Furuta, H. (1993) *Cellular and Molecular Biology of the Renin-Angiotensin System* (CRC, Boca Raton, FL).