Expression of Constitutively Active Angiotensin Receptors in the Rostral Ventrolateral Medulla Increases Blood Pressure

Andrew M. Allen, Jaspreet K. Dosanjh, Marco Erac, Sashikala Dassanayake, Ross D. Hannan, Walter G. Thomas

Abstract—Angiotensin type 1A (AT_{1A}) receptors are expressed within the rostral ventrolateral medulla, and microinjections of angiotensin II into this region increase sympathetic vasomotor tone. To determine the effect of sustained increases in AT_{1A} receptor density or activity in rostral ventrolateral medulla, we used radiotelemetry to monitor blood pressure in conscious rats before and after bilateral microinjection into the rostral ventrolateral medulla of adenoviruses encoding the wild-type AT_{1A} receptor or a constitutively active version of the receptor (Asn^{111}Gly, [N111G]AT_{1A}). The constitutively active receptor signals in the absence of angiotensin II. Adenovirus-directed receptor expression was extensively characterized both in vitro and in vivo. We established that adenoviral infection was limited to the rostral ventrolateral medulla and that receptor expression was sustained for ≥10 days; we also observed that adenoviral transgene expression occurs in glia, with no transgene expression observed in neurons of the rostral ventrolateral medulla. Rats receiving the wild-type AT_{1A} receptor showed no change in blood pressure, whereas animals receiving the [N111G]AT_{1A} receptor displayed an increase in blood pressure that persisted for 3 to 4 days before returning to basal levels. These data indicate that increased AT_{1A} receptor activity (not just overexpression) is a primary determinant of efferent drive from rostral ventrolateral medulla and reveal counterregulatory processes that moderate AT_{1A} receptor actions at this crucial relay point. More importantly, they imply that constitutive receptor signaling in glia of the rostral ventrolateral medulla can modulate the activity of adjacent neurons to change blood pressure. (Hypertension. 2006;47:1054-1061.)

Key Words: receptor, angiotensin ■ rats, inbred WKY ■ blood pressure

Neurons of the rostral ventrolateral medulla (RVLM) play a critical role in the generation and regulation of sympathetic vasomotor tone and in maintaining resting blood pressure. Efferent information from the RVLM is carried by a population of bulbospinal, glutamatergic neurons that project monosynaptically to the sympathetic preganglionic neurons located in the intermediolateral cell column of the spinal cord. At least 2 populations of these RVLM neurons have been identified: catecholaminergic C_{1} cells and non-C_{1} cells. Both groups of neurons potentially contribute to tonic and reflex regulation of sympathetic vasomotor tone. Angiotensin II (Ang II) is a peptide hormone with actions on the heart, blood vessels, adrenal gland, kidney, and brain that regulate arterial blood pressure and fluid and electrolyte homeostasis. Within the central nervous system (CNS), Ang II is one of multiple neurotransmitters and neuromodulators that act in the RVLM. Microinjection of Ang II into the RVLM induces a sympathetically mediated increase in blood pressure through activation of angiotensin type 1A (AT_{1A}) receptors. Angiotensin AT_{1A} receptors are expressed in the RVLM of most mammalian species studied, including humans. Although in vitro recordings indicate that Ang II can directly stimulate the RVLM premotor neurons, the precise cellular distribution of angiotensin type 1 receptors (AT_{1R}) in the RVLM has not been delineated. Indeed, AT_{1R} are expressed in cultured, purified rat brain astrocytes, and the effects of Ang II on neuronal function may involve the activation of non-neuronal AT_{1R}. Thus, the cellular context in which Ang II modulates activity in the RVLM is unclear.

In this study, we observed that adenoviral-induced transgene expression, under the control of the cytomegalovirus promoter (CMV), was only detected in glial cells within the RVLM. Thus, we investigated the effect on blood pressure of modulating the expression and/or activity of AT_{1A} receptors in glia in the RVLM, using adenoviral directed–expression of the wild type and a mutated version of the AT_{1A} receptor (Asn^{111}Gly, [N111G]AT_{1A}), which displays the unique capacity to signal constitutively in the absence of Ang II binding and stimulation. We report here that glial expression of the [N111G]AT_{1A} receptor in the RVLM, but not the wild-type receptor, raised blood pressure in conscious rats, indicating that increased receptor activity (and not mere overexpression) is required to drive efferent pathways. Despite sustained
levels of [N111G]AT1A receptor expression in RVLm, this elevated blood pressure returned toward control levels by post-injection day 4, signifying the presence of important counterregulatory processes that dampen AT1R actions at this crucial relay point for sympathetic vasomotor tone.

Methods

Generation of Adenoviruses

An adenovirus encoding the wild-type rat AT1A receptor (AdNHA-AT1A) has been previously described13; its titer was 0.64×10^{12} virus particles (VP)/mL. Mutation of asparagine131 to glycine in the third transmembrane domain of the AT1A receptor ([N111G]AT1A) produces constitutive activity. An adenovirus encoding [N111G]AT1A (AdNHA[N111G]AT1A) was produced by subcloning from pRc/CMV/N111GAT1A15 into pAdTrack-CMV shuttle vector, recombination with pAdEasy-1, and amplification and purification as previously described13,16; the titer was 0.32×10^{12} VP/mL. Both the wild-type and [N111G]AT1A receptors carry an N-terminal HA epitope tag (YPYDVPDYA) for detection of expression, and both viruses include a separate CMV promoter driving expression of green fluorescence protein (GFP). The control AdG8, adenovirus expresses no transgene other than a CMV-driven GFP; the titer of AdGo was 0.81×10^{12} VP/mL.

Adenoviral Infection of HEK293 Cells

HEK293 cells (obtained from the American Type Culture Collection, Manassas, VA), a well characterized cell line with low levels of endogenous AT1A receptor expression, were cultured in DMEM containing fetal bovine serum (10%), penicillin G sodium (100 μg/mL), streptomycin sulfate (100 μg/mL) and amphotericin B (0.25 μg/mL). For experiments, the cells were subcultured in 12-well plates (coated with 0.1% gelatin) at ×10{sup 5} cells/well and infected with AdNHA-AT1A (1.6×10{sup 6} VP) 48 hours after plating. For in vitro studies the adenoviral concentration is defined as the number of virus particles added per cell, the multiplicity of infection (MOI). For the binding and inositol phosphate assays, the adenoviruses were used at an MOI of 300. For the Western blotting, cells were infected with the adenoviruses at MOI in the range of 1 to 100. At 48 hours post-infection, >90% of the cells displayed GFP fluorescence, confirming viral infection.

Previously described methods were used for preparation of cell extracts and Western blotting, determination of cell surface receptor expression and affinity, and wild-type and mutant receptor-induced generation of inositol phosphates in response to Ang II stimulation.15

In Vivo Studies

All experiments were performed in accordance with the Australian National Health and Medical Research Council “Code of Practice for the Care and Use of Animals for Scientific Purposes” and were approved by the Institutional Animal Experimentation Ethics and Biosafety committees. Male Wistar Kyoto (WKY) rats (280 to 300 g), obtained from the Animal Resource Centre (Canning Vale, West Australia, Australia), were housed at constant temperature (22±1°C) on a 12-hour light:dark cycle with ad libitum access to standard rat chow and water.

Microinjections Into the Cerebral Cortex

To determine the timecourse and density of adenoviral-induced receptor expression, microinjections (50 nL) of AdNHA-AT1A were made, using standard methods,19 into the cerebral cortex (a site containing undetectable levels of endogenous AT1A receptor expression) of rats anesthetized with sodium pentobarbitone (Nembutal, 60 mg/kg IP). In 11 rats, different AdNHA-AT1A virus titers (6.4×10{sup 6}, 3.2×10{sup 6}, 1.6×10{sup 6}, and 0.64×10{sup 6} VP in 50 nL) were injected and AT1 receptor density determined 3 days later by quantitative in vitro autoradiography. In 13 rats, AdNHA-AT1A (1.6×10{sup 6} VP in 50 nL) was microinjected into the cortex, and AT1 receptor density determined after 1, 3, 7, and 10 days.

Quantitative In Vitro Autoradiography

The density of AT1A receptor binding was determined using standard methods using 125I-[Sar1,Ile8]Ang II as radioligand. AT1 receptor density was defined by displacement of radioligand binding with 1 μmol/L candesartan (Astra Hässle, Sweden).

Microinjections Into the RVLM

The position of the RVLM was determined by antidromic mapping of the facial nucleus. Four 50-nL microinjections of adenovirus (1.6×10{sup 6} VP/50 nL) were made into the RVLM, 1.8-mm lateral to the midline, 0.3- and 0.7-mm caudal to the caudal pole of the facial nucleus at 2 depths, defined by the ventral edge of the facial nucleus and separated by 0.4 mm. Each injection was made over 30 to 60 s and the pipette left in place for ~2 minutes before moving to the next position. For the in vivo blood pressure experiments, AdNHA-AT1A, AdNHA[N111G]AT1A, and AdG8 were injected bilaterally, whereas for cellular expression studies unilateral injections were made.

Verification of Microinjection Sites

Rats were perfused, via the left ventricle, with saline followed by 4% paraformaldehyde. The brains were removed, post-fixed for 2 hours, immersed in 20% sucrose in 0.1 mol/L phosphate buffer (pH 7.4) overnight, and then frozen for sectioning. Serial 40-μm sections were cut on a freezing microtome, mounted onto gelatin-coated glass slides, and coverslipped using buffered glycerol. Sites of microinjection were localized by examining expression of GFP under a fluorescence microscope.

Immunohistochemistry

Alternative sections from those used for microinjection verification were used for immunohistochemistry. The primary antibodies used were mouse anti-dopamine β-hydroxylase (DBH) 1:500; Chemicon Int, Temecula, CA), mouse antineuN (1:500; Chemicon Int), rabbit anti-gial fibrillary acidic protein (GFAP) 1:500; DakoCytoimm, France) or rabbit anti-AT1A receptor. Secondary antibodies were purchased from Vector Laboratories, Burlingame, CA.

Blood Pressure Telemetry

Rats (n=18) were anesthetized with sodium pentobarbitone (≥60 mg/kg, IP) and telemeters (PAC40, Transoma Medical, St. Paul, MN) were implanted, with the catheter in the abdominal aorta. The rats were allowed to recover for ≥10 days, and blood pressure was then measured daily between 15:00 and 16:00 hours (well before the commencement of the dark phase at 19:00) for 7 days (500 Hz sampling rate). DATAQuest A.R.T. 3.0, Transoma Medical, St. Paul, MN). The blood pressure telemeters were activated ≥30 minutes before commencement of the recording period, and the animals remained undisturbed in their home cage. On day 8, the rats were injected bilaterally in the RVLM with adenovirus as described above. Blood pressure was then measured, at the same time, for another 7 days. At completion of the recording period the rats were killed by perfusion and brains examined to localize the injection site.

Analysis

All statistical analysis was performed using Minitab version 14 (Minitab, State College, PA).

Quantitative In Vitro Autoradiography

Density of AT1A receptor binding was determined using standard methods.8 Radioactive standards (125I) were purchased from Amersham Biosciences, UK Computer analysis of image density used Scion Image 4.0.2 (Scion Corporation, Frederick, MD). To enable comparison, sections containing the nucleus of the solitary tract, at the level of the caudal pole of the area postrema, were incubated in each experiment. Values were then normalized to the level of endogenous AT1A receptor expression in this nucleus. One-way analysis of variance, followed by all pairwise multiple comparison
Characterizing AT1A Receptor Adenoviruses

To confirm the functional characteristics of receptors expressed by AdNHA-AT1A and AdNHA[N111G]AT1A, we performed Western analyses, competitive binding assays, and IP accumulation assays (Figure 1). Increasing the amount of AdNHA-AT1A (MOI, 0 to 30) increased receptor expression (Figure 1A), which resolved as a broad migrating band ranging between 50 to 200 kDa that represents the mature receptor protein glycosylated at multiple sites and a band of approximately 35 kDa, the unmodified form of the receptor, as previously reported. At an MOI of 10, the [N111G]AT1A receptor also displayed similar banding patterns and approximately equivalent expression compared with the wild-type receptor (Figure 1A).

To verify that the [N111G]AT1A receptor is constitutively active, we measured the accumulation of inositol phosphates (a hallmark of Gq/11-coupled signaling). Cells expressing the [N111G]AT1A receptor displayed an enhanced basal level of inositol phosphate generation in the absence of Ang II stimulation (Figure 1B). In accordance with previous reports, this increased basal activity represents a 25% increase above the unstimulated wild-type receptor, which equates to ~40% of maximal inositol phosphate accumulation observed following Ang II stimulation of the wild-type receptor.

When expressed in HEK293 cells, both AdNHA-AT1A and AdNHA[N111G]AT1A receptors displaced [125I]Ang II with increasing concentrations of unlabeled Ang II, revealing similar high receptor affinities (Kd = 0.4 nM for the wild-type receptor; Kd = 1.4 nM for the mutant receptor [Figure 1C]) as reported previously.

Expression of Wild-Type or [N111G]AT1A Receptors In Vivo

Microinjections of different titers of AdNHA-AT1A into the cerebral cortex resulted in expression of AT1R in a longitudinally oriented ellipse. Binding density was measured in sections from the middle of the ellipse to ensure that they were taken from a region of uniform receptor density. Increasing concentration of virus resulted in significant increases in the receptor binding density per unit area (Figure 2A). Microinjection of 1.6 x 10⁶ VP/50 nL resulted in a receptor density comparable to that of endogenous AT1 receptor expression in the nucleus of the solitary tract (NTS). This concentration was used for all further experiments.

One day after microinjection of 1.6 x 10⁶ virus particles into the cerebral cortex, AT1R expression was 42% (SD 5) of the endogenous level found in the NTS (Figure 2B). Maximal levels were obtained by day 3, and these did not change significantly at day 7 or 10. Following microinjection of AdNHA[N111G]AT1A into the RVLM, binding to the [N111G]AT1A receptor in the RVLM was similar on day 3 and 7 (44 [SD 15] dpm/mm² [day 3] versus 68 [SD 17] dpm/mm² [day 7]; n = 2 rats per time point) (Figure 2C and 2D). This represents an ~7-fold increase in AT1R density over endogenous AT1R expression in the RVLM (7.5 [SD 5] dpm/mm²) and a similar level of expression to that of the

Blood Pressure Telemetry

Mean arterial pressure (MAP) levels were examined for all rats in which bilateral microinjections occurred in the RVLM. Two-way analysis of variance with repeated measures (general linear model) indicated that a significant difference occurred between the MAP values of the AdG, animals compared with the other 2 groups during the control period. Thus, the data were transformed to indicate change in blood pressure with the control value being taken as the mean of 3 days before injection. Each data point was then expressed as (observed value−control value/control value) x 100. These values were then compared by 2-way analysis of variance with repeated measure (general linear model) with Tukey post hoc test used for pairwise comparison. Data for systolic and diastolic blood pressures and heart rate were also examined for the rats that received bilateral RVLM microinjection of AdNHA[N111G]AT1A and statistically analyzed by 1-way analysis of variance for repeated measures with Tukey post hoc test.

Figure 1. Characterization of adenoviral-induced AT1A receptors in vitro. HEK 293 cells were infected with AdNHA-AT1A (0 to 30 MOI) and Western blotted with anti-HA antibody (A). Receptor expression induced by AdNHA[N111G]AT1A (10 MOI) is shown for comparison. HEK293 cells infected with AdNHA[N111G]AT1A (~3 MOI) show increased basal inositol phosphate accumulation compared with AdNHAAT1A (also ~3 MOI); maximal Ang II-induced inositol phosphate accumulation was similar for the 2 receptors (B). Both receptors also showed an equivalent binding affinity for Ang II (C).
endogenous AT$_1$R in the NTS measured in the same experiment (64 $\pm$ 22 dpm/mm$^2$).

**Cellular Expression of Adenoviral Transgene in the RVLM In Vivo**

Following injection into the RVLM, the different adenoviral constructs showed an identical pattern of GFP expression. Spread of the adenoviral infection from the region of injection was minimal and resulted in coverage of the RVLM from the facial nucleus for $\approx$1-mm caudal (Figure 3).

Following correctly placed microinjections, GFP expression was observed surrounding DBH-positive neurons (Figure 4). Despite systematic evaluation of all infected regions in all of the animals included in this study, cellular colocalization of these markers was never observed (Figure 4). Colocalization of GFP and AT$_1$R-like immunoreactivity was always observed, with the GFP predominantly localized to the soma and proximal processes whereas the AT$_1$R-like immunoreactivity extended into more distal processes (Figure 5A). Within the RVLM, no colocalization with the neuronal microtubular protein, NeuN, was observed (Figure 5B). Morphological examination indicated that GFP expression was predominantly in glia (Figure 5A, 5C, and 5D), and this

**Figure 2.** Characterization of the density and time-course of AT$_{1A}$ receptor expression in vivo. AT$_{1A}$ receptors were quantified using in vitro autoradiography after microinjection of AdNHA-AT$_{1A}$ into the rat cerebral cortex (A). Receptor density was normalized to endogenous AT$_1$R in the nucleus of the solitary tract. Significant differences were obtained using ANOVA and Student–Newman–Keuls all pairwise multiple comparison. The timecourse of expression was determined following microinjections of $1.6 \times 10^8$ VP/50 nL into the cerebral cortex (B). Asterisks indicate significant differences compared with the density at day 1. Timecourse of expression for the [N111G]AT$_{1A}$ receptor in the RVLM at days 3 (C) and 7 (D) after microinjection. Cb indicates cerebellum.

**Figure 3.** Localization of transgene expression in the RVLM. Blood pressure analysis was performed on conscious rats in which adenoviral injections were bilaterally confined to the RVLM. The boxed regions in the schematic coronal section of the rat medulla oblongata at the level of the RVLM (11.96-mm caudal to bregma) (A) correspond to the photomicrographs in (B) and (C). These show correctly placed microinjections of AdNHA[N111G]AT$_{1A}$ within the left (B) and right (C) RVLM. GFP expression (white) is seen in cell bodies and processes within the RVLM; scale bar depicts 200 $\mu$m. 4V indicates fourth ventricle; sol, solitary tract; sp5, spinal trigeminal tract; py, pyramidal tract; ml, medial lemniscus; Amb, nucleus ambiguus; ROb/RPa, raphé obscurus/pallidus; IOD/IOM, dorsal and medial divisions of the inferior olivary nucleus.
was confirmed for a subgroup of cells with GFAP immuno-reactivity being colocalized in \( \approx 30\% \) of GFP-labeled cells (Figure 5C and 5D).

**Effect of Adenoviral Transduction in the RVLM on Blood Pressure in Conscious Rats**

Bilateral microinjections of adenoviruses were made into the RVLM of 18 male WKY rats, which had been previously implanted with blood pressure telemeters. Post hoc analysis of the distribution of GFP expression indicated that these injections were accurately placed (bilaterally in the RVLM located between the compact formation of the nucleus ambiguus and the ventral brain surface and within 1 mm of the caudal pole of the facial nucleus) in 5 AdNHA[N111G]AT\(_{1\alpha}\), 4 AdNHA-AT\(_{1\alpha}\), and 4 AdGo microinjected rats (Figure 3). Only these animals were included in further analysis of the blood pressure responses.

In the pre-injection period, basal blood pressure of the AdGo group was significantly lower than that of the other 2 groups (Figure 6A). There is no reason for this difference as the rats were randomly assigned to each group and studied, in groups of 8 animals, concurrently. For statistical analysis, the post-injection blood pressure measurements were normalized relative to a 3-day control period just before injection (days 5, 6, and 7). Thus, results are also expressed as % change in blood pressure (Figure 6B).

Microinjection of AdNHA[N111G]AT\(_{1\alpha}\) bilaterally into the RVLM induced a significant increase in mean arterial blood pressure for 3 to 4 days, when compared with the AdNHA-AT\(_{1\alpha}\) and AdGo injected groups (Figure 6A). There was no significant difference between the groups from day 5 onward. The increase in MAP in the AdNHA[N111G]AT\(_{1\alpha}\) rats was attributed to an increase in both systolic and diastolic blood pressures over the same timecourse. A tendency toward an increase in heart rate was also observed for the 3 days after microinjection of AdNHA[N111G]AT\(_{1\alpha}\) but, because of the large variance, this did not attain significance (control period: 296 [SD 14]; day 3 post AdNHA[N111G]AT\(_{1\alpha}\) injection: 322 [SD 36] \(P=0.19\)). No significant change in blood pressure, when compared with the control period or between each other, was observed for the AdNHA-AT\(_{1\alpha}\) or AdGo groups.

**Discussion**

Bilateral microinjection into the RVLM of an adenovirus, which expresses a constitutively active version of the AT\(_{1\alpha}\) receptor in glial cells, increases MAP in conscious, freely moving rats of a normotensive strain. Expression of similar levels of the wild-type AT\(_{1\alpha}\) receptor, or the control adenovirus with no receptor transgene, did not affect blood pressure. Although not directly measured in this study, we
suggest that the increase in blood pressure following micro-
jection of AdNHA[N111G]AT1A into the RVLM would be
caused by increased sympathetic vasomotor tone.

Virally-derived proteins, including specifically the ectopic
expression of the AT1A receptors, were only detectable in glia
of the RVLM, indicating that a change in activity of a G
protein-coupled receptor in glia has the capacity to alter blood
pressure. Glia were initially considered to play only a supporting
role within the CNS, but recent work has demonstrated that
considerable interactions occur between glia and neurons,
resulting in altered neuronal signaling.25 Although the mech-
anism behind the observation in this study remains to be
determined, we propose that modulation of glial angioten-
sinogen synthesis and release might be involved. Glia are
the primary source of angiotensinogen (the obligate precursor
for Ang II) in the brain,26,27 including in the RVLM.28 Although
not tested in glia, it is known that angiotensinogen synthesis
is modulated by Ang II acting through AT1R.29,30 Hence, one
possibility is that enhanced AT1A receptor activity in glia may
upregulate local angiotensinogen synthesis. This in turn could
activate the endogenous AT1R, which occurs on RVLM
presympathetic neurons, to increase sympathetic vasomotor
activity.

Alternatively, and despite the clearly enhanced glial trans-
gene expression, we cannot rule out the possibility that
transgene expression is occurring in other cell types, includ-
ing neurons, of the RVLM, but at a level that is not detectable
using the techniques described. In some of the rats from this
study, where injections occurred outside the RVLM, we
observed adeno-viral transgene expression in large neurons in
the facial nucleus and compact formation of the nucleus
ambiguous (data not shown). However, despite systematic
observation we could not detect transgene expression in
neurons of the RVLM. Others have also reported that aden-
viral transgene expression, driven by the CMV promoter,
results in expression predominantly within glia following
central administration.31 Indeed, in vitro experiments have
also indicated that the CMV promoter is not effective at
inducing transgene expression in some neurons.32 Whether,
in the case of RVLM neurons, this reflects the absence of
appropriate transcription factors or involves active silencing
of the CMV promoter is not known. In addition, altered
expression in vascular endothelial cells must be considered.
Within the nucleus of the solitary tract, Ang II is proposed to
alter neuronal activity via activation of endothelial AT1Rs to
modulate NO synthase activity.11 Cardiovascular effects fol-
lowing adeno-viral-induced expression of NO synthase, under
the control of the CMV promoter, in the RVLM have also
been demonstrated.33 However, in our study no transgene
expression in cells with morphological characteristics of
endothelial cells was observed, although this was not system-
atically studied. What is clear in this study is that the
predominant increase in AT1A receptor expression is occur-
ing within glia and that the level of ectopic expression in
neurons or other cells, if any, represents a very small proportion
of the total within the RVLM and offers no increase over that of
the endogenous receptor in this nucleus.

The renin–angiotensin system is one of the most widely
studied homeostatic, hormonal systems and extensive site-
directed mutagenesis of its principal receptor, the AT1 recep-
tor, has yielded a number of interesting receptor variants that
display unique properties. Among these, mutation at Aspara-
gine31, a highly conserved residue in the third transmembrane-
spanning domain of the receptor involved in Ang II ligand
binding, produces a receptor ([N111G]AT1A) receptor with
constitutive activity12 (ie, the capacity to activate signaling in
the absence of ligand binding). Such mutants represent unique tools
for examining the complex biology of Ang II/AT1 receptors in
vivo. Delivery of [N111G]AT1A using adeno-virus into specific
brain nuclei, as we have described in the present study,
permits us for the first time to recapitulate the activation of
the renin–angiotensin system (ie, an increased production and
access of Ang II to enhanced levels of AT1A receptors) but in
a manner that negates the needs to constantly infuse/provide
Ang II stimulation. The advantage of such an approach is that
direct comparisons can be made with the wild-type receptor
(which requires Ang II stimulation to become activated and is
also rapidly desensitized) and can lead to conclusions, such as
the major one from this study, that it is the increased AT1A
receptor activity in the RVLM, rather than density, that
determines increased blood pressure responses.

The capacity to “switch-on” the renin–angiotensin system
by delivering a constitutive receptor in such a discrete manner
is important, because several groups have demonstrated that
there is little, if any, tonic activity exerted through the AT1A
receptor in the RVLM of normotensive rats. In anesthetized
normotensive rats, microinjection of non-peptide AT1R an-
tagonists, such as candesartan, have no effect on resting
blood pressure or sympathetic vasomotor activity,19 whereas,
in conscious rats, a small increase in blood pressure is observed following microinjection of another antagonist, losartan. These published results have been used to support the view that Ang II inputs to the RVLM have little effect on basal regulation of blood pressure. Only after activation of specific pathways, such as that from the hypothalamic paraventricular nucleus, is a response to AT1R antagonism observed in the RVLM of normotensive rats. An obvious extension of the current studies will be to examine whether the blood pressure responses to a stimulus that might be expected to activate central Ang II activity, such as dehydration or administration of hypertonic saline, are enhanced following microinjection of the wild-type AT1A receptor adeno-virus into the RVLM.

Despite persistent receptor expression in the RVLM (≥10 days), the blood pressure increase in response to increased AT1A receptor activity was only sustained for 3 to 4 days after injection. There is no sign of localized damage or infection at the site of the injection to indicate that this is a nonspecific effect. Indeed, the animals injected with viruses that were identical except for the minor change in transgene (AdNHA-AT1A) or deletion of a transgene (AdGΔ) showed no appreciable change in their blood pressures throughout the period of the experiment. In addition, none of the animals showed any behavioral changes indicative of nonspecific effects in the brain. These observations support the contention that the pressor response is caused by the increased AT1A receptor activity. There are several plausible explanations for the transient nature of the blood pressure change. Although we demonstrate increased intracellular inositol phosphate signaling in vitro following infection with AdNHA[1111G]AT1A, it is not possible to perform these assays in vivo and thereby confirm sustained constitutive signaling for the duration of the increased receptor protein expression. Accordingly, we cannot rule out an intracellular response to downregulate or dampen the increased signaling. Similarly, other cellular homeostatic responses to either the increased activity or elevated blood pressure might occur. In response to a sustained stimulatory signal from neighboring astrocytes, it is possible that changes in neuronal responsiveness might occur to restore “normal” levels of activity. Both activity-dependent and -independent changes in neuronal gene expression have been reported in response to experimental manipulation of cell activity. Finally, and just as likely, we may be observing a homeostatic response of the organism via one of the many complex feedback loops that occur in the long-term regulation of blood pressure.

Perspectives
We have demonstrated that increased AT1A receptor activity in the RVLM leads to an increase in blood pressure that is sustained for several days. This increase in receptor activity is predominantly occurring in glia, suggesting that altered receptor-mediated signaling in these cells within the RVLM can modulate blood pressure. Our hypothesis is that this is through increased local production of Ang II, which excites the bulbospinal, sympathetic premotor neurons to increase sympathetic vasomotor activity. While the response is sustained for several days, homeostatic mechanisms restore blood pressure to resting levels in the face of prolonged receptor activity. Together these results demonstrate that adenoviral-induced expression of transgenes in the RVLM is a powerful means of increasing our understanding of glial-neuronal interactions in vivo as well as homeostatic mechanisms involved in regulation of blood pressure.

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