DISTRIBUTION OF ANGIOTENSIN TYPE 1A RECEPTOR-CONTAINING CELLS IN THE BRAINS OF BACTERIAL ARTIFICIAL CHROMOSOME TRANSGENIC MICE


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Abstract—In the central nervous system, angiotensin II (AngII) binds to angiotensin type 1 receptors (AT1Rs) to affect autonomic and endocrine functions as well as learning and memory. However, understanding the function of cells containing AT1Rs has been restricted by limited availability of specific sera, difficulties discriminating AT1R-immunoreactive cells in many brain regions and, the identification of AT1R-containing neurons for physiological and molecular studies. Here, we demonstrate that an Agtr1a bacterial artificial chromosome (BAC) transgenic reporter mouse line that expresses type A AT1Rs (AT1aRs) identified by enhanced green fluorescent protein (EGFP) overcomes these shortcomings. Throughout the brain, AT1aR-EGFP was detected in the nuclei and cytoplasm of cells, most of which were neurons. EGFP often extended into dendritic processes and could be identified either natively or with immunolabeling of GFP. The distribution of AT1aR-EGFP cells in brain closely corresponded to that reported for AngII binding and AT1aR protein and mRNA. In particular, AT1aR-EGFP cells were in autonomic regions (e.g., hypothalamic paraventricular nucleus, central nucleus of the amygdala, parabrachial nucleus, nuclei of the solitary tract and rostral ventrolateral medulla) and in regions involved in electrolyte and fluid balance (i.e., subfornical organ) and learning and memory (i.e., cerebral cortex and hippocampus). Additionally, dual label electron microscopic studies in select brain areas demonstrate that cells containing AT1aR-EGFP colocalize with AT1R-immunoreactivity. Assessment of AngII-induced free radical production in isolated EGFP cells demonstrated feasibility of studies investigating AT1aR signaling ex vivo. These findings support the utility of Agtr1a BAC transgenic reporter mice for future studies understanding the role of AT1-R-containing cells in brain function. © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: autonomic nuclei, hypothalamus, subfornical organ, amygdala, nucleus of the solitary tract, rostral ventrolateral medulla.

INTRODUCTION

Angiotensin type 1 receptors (AT1Rs) mediate regulation of autonomic function by angiotensin II (AngII) in the CNS. In particular, brain AT1Rs regulate blood pressure, fluid and electrolyte balance and neuroendocrine functions (Allen et al., 2000; McKinley et al., 2003; Zimmerman et al., 2004). Moreover, AT1Rs are involved in learning and memory processes (Denny et al., 1991; Wright et al., 1993; Belcheva et al., 2000) as well as regulation of inflammation (Benicky et al., 2011). Previous anatomical studies have shown that AngII binding and AT1R-immunoreactivity (ir) and mRNA are expressed in brain regions that are known to regulate these functions. These include the subfornical organ (SFO), paraventricular nucleus (PVN) of the hypothalamus, amygdala, hippocampus, nucleus of the solitary tract (NTS) and rostral ventrolateral medulla (RVLM) (Rowe et al., 1990; Tsutsumi and Saavedra, 1991; Aldred et al., 1993; Lenkei et al., 1997; Hauser et al., 1998).

Characterizing the phenotype and understanding the function of AT1R-containing cells in the CNS has been

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restricted by several factors. First, the availability of specific AT-R antibodies is limited. Second, since immunoreactivity for AT₁R may be relegated to certain organelles or cellular regions (Pierce et al., 2009), distinct identification of AT₁R-immunoreactive cells is difficult in many brain regions. Third, detection of AT₁R-ir for some antibodies is optimal in brain tissue that is perfusion-fixed with paraformaldehyde containing acrolein (Huang et al., 2003; Glass et al., 2005; Pierce et al., 2009). Unfortunately, acrolein can auto-fluoresce making it difficult to visualize cells with low levels of AT₁Rs with immunofluorescence. Fourth, understanding the function of cells containing AT₁Rs has been limited due to lack of identification of these cells in live preparations in which physiological and/or molecular phenomena can be observed.

In this study, we describe an Agtr1a bacterial artificial chromosome (BAC) transgenic reporter mouse line obtained from the Gene Expression Nervous System Atlas (GENSAT) project (Gong et al., 2003) to overcome the shortcomings. Cells containing enhanced green fluorescent protein (EGFP) indicative of type A AT₁R (AT₁aR) expression were detected throughout the brain. Light and electron microscopic studies confirmed that nearly all of AT₁aR-EGFP cells were neurons. Vital or aldehyde-fixed EGFP-containing cells could be identified either natively supporting their utility for physiological and molecular studies or immunocytochemically supporting their utility for light and electron microscopic studies. The distribution of AT₁aR-EGFP cells throughout the brain was consistent with that shown previously for AngII binding, AT₁R-ir and mRNA. Moreover, electron microscopy showed colocalization of AT₁aR-EGFP and AT₁-R-ir in cells supporting the fidelity of the Agtr1a BAC transgenic reporter mouse line for use in future studies.

**EXPERIMENTAL PROCEDURES**

**Animals**

All methods were conducted in accordance with the 2011 Eighth Edition of the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Rockefeller University Institutional Animal Care and Use Committee. Adult male and female Agtr1a BAC transgenic mice and their non-transgenic littersmates (N = 30; see below for description) were housed in a reversed light cycle (12:12 light:dark cycle, lights on at 6 pm) temperature-controlled room with free access to food and water. Mice were between 2 and 3 months old in the anatomical studies and between 3 and 5 weeks old in the physiological studies at the time of sacrifice. The estrous cycle stage of female mice was monitored for two weeks prior to physiological studies at the time of sacrifice. The estrous cycle phenomena can be observed.

**Antisera.** All antisera have been well characterized. An affinity purified rabbit antibody to the AT₁R (92578) was raised against the C terminal portion (amino acids 341–355; PDB-NMSSAATKPC) of the rat AT₁aR receptor (note: the C terminal portion of the mouse differs by one amino acid – PDB-NMSSAATKPC). The antiserum is the same used in earlier studies (Huang et al., 2003; Glass et al., 2005) except that it was not purified for separation of the AT₁a and AT₁b subtypes, and is thus designated as an AT₁R antiserum (Pierce et al., 2009). The selectivity of this antiserum for AT₁ versus AT₂ receptors has been demonstrated using Chinese hamster ovarian cells differentially transfected with AT₁aRs, AT₁bRs and angiotensin type 2 receptors (AT₂Rs) (Huang et al., 2003). Preadsorption of the antibody (1:600 dilution) with the antigenic peptide (100 μg/ml) completely removed punctate immunolabeling in the NTS and area postrema (AP) in rat brain sections fixed with acrolein and paraformaldehyde (Huang et al., 2003). To additionally test the specificity of the antibody, AT₁R immunolabeling was performed in mice with conditional deletion of AT₁aR in the SFO. For this, adult male mice
harboring a "floxed" AT1R allele (kind gift of Dr. Thomas M. Coffman) (Gurley et al., 2011) underwent SFO-targeted injection into the lateral ventricle of a recombinant adenovirus (Ad) expressing Cre-recombinase (Ad-Cre; 3 x 10^8 pfu/ml; 500 nL; N = 4) or liter-matched control virus Ad-LacZ (N = 4) over a period of 3–5 min, as described previously (Sinnayah et al., 2004). Ten days later, mice were perfused with acrolein/parafomaldehyde and processed for AT1R peroxidase immunocytochemistry as described below. Intense peroxidase immunoreaction product was seen in the SFO of Ad-LacZ-injected mice but not in Ad-Cre-injected mice (Fig. 1).

The chicken green fluorescent protein (GFP) antibody was generated against recombinant GFP (Aves Lab Inc., San Diego, CA) and recognizes the gene product of EGFP-expressing transgenic mice (Encinas et al., 2006). The specificity of this antibody has been demonstrated by Western blot (see data sheet for EGFP-1020 at www.aveslab.com) and has been used in our previous studies (Bulloch et al., 2008; Milner et al., 2010). Moreover, this antibody does not label cells in brain tissue from mice lacking EGFP (Volkmann et al., 2010; also see Fig. 1).

A polyclonal antibody to tyrosine hydroxylase (TH) generated in sheep (#AB1542; Millipore Corporation, Bedford, MA) was used. This antibody recognizes a single band (~60 kDa) on Western blot from PC12 cells stimulated with okadaic acid (manufacturers data sheet). The specificity is supported by a neuroanatomical localization consistent with that known for noradrenaline (Noack and Lewis, 1989; Kaufling et al., 2009). Moreover, it labels the same brain structures as polyclonal rabbit anti-TH (Chemicon, AB152; Ramer, 2008).

A polyclonal antibody to arginine vasopressin (AVP) was generated in guinea pig (T-5048, lot #061305; Peninsula Laboratories Inc., San Carlos, CA) and has been used in recent studies (Coleman et al., 2009; Milner et al., 2010). By radioimmunoassay, this antiserum shows complete recognition of vasopressin, a slight cross-reaction (<1%) with other vasopressin derivatives and no recognition of oxytocin (Peninsula Laboratories). The specificity of this antibody has been confirmed by preadsorption immunocytochemistry, in which all staining is abolished (see Hundahl et al., 2010), and by the absence of staining in the Brattleboro rat, which due to a natural genetic mutation is unable to produce AVP (Drouyer et al., 2010).

A mouse monoclonal antibody to glial fibrillary acidic protein (GFAP) was purchased from Cell Signaling Technology (GA5 #3670; Boston, MA). This antibody recognizes a single 52-kDa band on Western blots, (see manufacturer’s data sheet) and yields a cellular morphology and distribution pattern in rat brain identical to known astrocyte distributions (see Levendusky et al., 2009).

**Immunoperoxidase methods.** Coronal sections (approximately 240 μm apart) through the rostrocaudal extent of the Agtr1a BAC transgenic mouse (or their non-transgenic littermates) brains were processed for the immunocytochemical localization of GFP using the avidin–biotin complex (ABC) method (Milner et al., 2011). A 24-h incubation at room temperature (~25 °C) was followed by a 24-h 4 °C incubation in

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**Fig. 1.** AT1R-ir is absent following a targeted SFO deletion of AT1aR. Intense peroxidase AT1R-ir was seen in the SFO of a control Ad-LacZ-injected AT1aR-floxed mouse (A) but not an Ad-Cre-injected AT1aR-floxed mouse (B). Bar = 0.1 mm.

**Fig. 2.** Examples of AT1aR-EGFP cells in the brains of Agtr1a BAC transgenic mice. (A–C) As shown in these examples from fixed tissue sections, GFP peroxidase reaction product is visible in light, dark and clusters of cells. (D–F) As shown in these examples from the PVN (D), NTS (E) and SFO (F), AT1aR-EGFP cells (green) overlapped, but did not colocalize, with GFAP-immunoreactive cells (magenta). Bar A–C = 50 μm; D = 40 μm; E and F = 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
the GFP antiserum (1:5000 dilution) in 0.1% BSA in Tris–saline (TS; pH 7.6). All sections then were incubated in a 1:400 dilution of biotinylated goat anti-chicken immunoglobulin (IgG) (Jackson ImmunoResearch Inc., West Grove, PA) BSA/TS for 30 min, rinsed in TS and incubated in ABC (at twice the recommended dilution; Vector Laboratories; Burlingame, CA) for 30 min. Sections then were reacted in 3,3′-diaminobenzidine (Sigma–Aldrich Chemical Co., Milwaukee, WI) and H2O2 in TS for 6 min, rinsed in PB, mounted on slides previously coated with 1% gelatin and dried in a desiccator. The slides were dehydrated through a graded series of ethanol and coverslipped from xylene with DPX (Sigma–Aldrich Chemical Co.).

Sections labeled for AT1aR-EGFP using immunoperoxidase were analyzed on a Nikon 80i light microscope equipped with bright-field and differential interference optics and a Microublisher digital camera (Q Imaging, Barnaby, British Columbia). The distribution of AT1aR-EGFP cells was plotted onto standardized brain maps (Hof et al., 2000) using Adobe Illustrator CS3 (Adobe systems). Each map represents the relative distribution of AT1aR-EGFP cells using a composite of 7 brains (4 female and 3 male). EGFP-containing cells were qualitatively divided into three groups: (1) light cells (open circles); (2) dark cells (solid circles); and (3) many dark cells (starbursts) (Fig. 2A–C). An additional set of sections was prepared for quantitative analysis. For this, from the rostrocaudal extent of 6 Agtr1a EGFP transgenic mice [3 males and 3 females (two in diestrus and one in estrus, both low estrogen states)] were punch coded, pooled into the same container processed using the same batch of primary and secondary antibodies (Pierce et al., 1999). AT1aR-EGFP cells from select brain regions then were counted from a 0.04-mm² area using a grid reticule on a Nikon Labophot microscope by a person blinded to experimental conditions. Differences between groups were determined using Student’s t-test.

Dual labeling immunofluorescence methods. Sections through select brain regions were dually labeled for EGFP and either AVP, TH or GFAP, using immunofluorescence as previously described (Gonzales et al., 2011). Briefly, sections were rinsed thoroughly in PB and incubated in 1% BSA in PB for 30 min. Sections then were placed in a cocktail of GFP antiserum (1:1000) and either the AVP (1:1200), TH antiserum (1:1000) or GFAP (1:1000) in 0.5% BSA/PB and 0.25% Triton-X 100 for 24 h at room temperature and for 24 h at 4°C. Next, sections were washed with PB and incubated for 1 h in a cocktail of Alexa Fluor 488 (green) goat anti-chicken IgG for demonstration of EGFP (1:400; Invitrogen-Molecular Probes, Carlsbad, CA) and either Texas Red 540 donkey anti-guinea-pig IgG for the demonstration of vasopressin (1:400; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), Alexa Fluor 647 (deep red) donkey anti-sheep IgG for demonstration of TH (1:400; Invitrogen-Molecular Probes) or Texas Red donkey anti-mouse IgG for demonstration of GFAP (1:400; Jackson) in 0.5% BSA/PB. Sections were washed in PB and then mounted on gelatin-coated slides, air-dried and coverslipped with ProLong mounting media (Invitrogen-Molecular Probes, Eugene, OR). Images were collected with a Leica TCS SP5 confocal microscope using an Argon laser (488) and a HeNe laser (543 and 633). Sequential scans were acquired to avoid any overlap between the wavelengths for the two labels. Z stack images (approximately 20 μm at 1 μm each) were taken and combined into a single image using the Leica application suite software.

Immunoelectron microscopic methods. To examine the morphology of AT1aR-GFP-containing cells, sections from select brain regions from 3 male Agtr1a BAC mice were processed for GFP immunoperoxidase as described for light microscopy. For the ultrastructural localization of AT1-R in Agtr1a BAC transgenic mice, sections through select brain regions were dual-labeled for AT1-R immunoperoxidase and GFF-immunogold as described previously (Milner et al., 2011). Briefly, sections were incubated in an AT1-R antiserum (1:1000), in 0.1% BSA in TS for 1 day at room temperature followed by an additional 2 days at 4°C. One day prior to further processing, GFF antiserum (1:5000) was added to the primary antibody diluent. To visualize AT1-R-ir, sections were incubated in biotinylated goat anti-rabbit IgG (Vector) and processed for the ABC peroxidase technique described above. To visualize GFF-labeling, sections were rinsed in TS and incubated in rabbit anti-chicken IgG conjugated to 1-nm gold particles (1:50, Electron Microscopy Sciences (EMS), Fort Washington, PA) in 0.01% gelatin and 0.08% BSA in 0.01 M phosphate-buffered saline (PBS; pH 7.4) at room temperature for 2 h. Sections were rinsed in PBS, post-fixed in 2% glutaraldehyde in PBS for 10 min, and rinsed in PBS followed by 0.2 M sodium citrate buffer (pH 7.4). The conjugated gold particles were enhanced by reaction in a silver solution (RPN491 Silver Enhance kit, GE Healthcare, Waukesha, WI) for 5 min.

Sections were post-fixed for 1 h in 2% osmium tetroxide, dehydrated through alcohols and propylene oxide, and embedded between two sheets of plastic in EMBed 812 (EMS). Ultrathin sections (70 nm thick) were cut from the region of interest 2A–C) using a Leica Ultracut UCT ultratome and collected on 400-mesh thin-bar copper grids (EMS). Sections were counterstained with uranyl acetate and Reynold’s lead citrate, and final processing, GFP antiserum (1:5000) was added to the primary antibody diluent. GFF-labeled sections were rinsed in PBS, post-fixed in 2% glutaraldehyde in PBS for 10 min, and rinsed in PBS followed by 0.2 M sodium citrate buffer (pH 7.4). The conjugated gold particles were enhanced by reaction in a silver solution (RPN491 Silver Enhance kit, GE Healthcare, Waukesha, WI) for 5 min.

Composite images of the distribution of AT1aR-EGFP cells and their corresponding immunogold localization were generated using Adobe Photoshop CS3 on a Macbook computer. On confocal images, far red (Alexa Fluor 647) images were pseudocolored magenta. Images were assembled into the final figures using Powerpoint 2008.

Reactive oxygen species (ROS) detection

Isolated cells were prepared from the PVN of juvenile (postnatal day 30–45 old) male Agtr1a BAC transgenic mice (N = 10) using previously described methods (Li et al., 1998; Wang et al., 2004).

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Male (N = 3)</th>
<th>Female (N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal caudate</td>
<td>38.2 ± 3.8</td>
<td>31.2 ± 3.7</td>
</tr>
<tr>
<td>SFO</td>
<td>45.8 ± 3.8</td>
<td>48.5 ± 3.6</td>
</tr>
<tr>
<td>PVN</td>
<td>40.8 ± 6.3</td>
<td>52.0 ± 5.2</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>36.0 ± 7.3</td>
<td>55.8 ± 4.2</td>
</tr>
<tr>
<td>NTS</td>
<td>28.5 ± 3.0</td>
<td>33.6 ± 5.0</td>
</tr>
<tr>
<td>RVLM</td>
<td>6.8 ± 0.7</td>
<td>5.2 ± 0.8</td>
</tr>
</tbody>
</table>
Fig. 3. Examples of electron microscopic immunoperoxidase localization of AT1aR-EGFP. (A) AT1aR-EGFP is found almost exclusively in neurons. The nucleus (N) of this neuron contains a nucleolus (nu). The cytoplasm contains numerous mitochondria (m), endoplasmic reticulum (er) and a Golgi apparatus (G). A dendrite (D) emanates from the soma. The neuronal somata is adjacent to the endothelial cell (En) of a blood vessel. (B) GFP-ir fills a dendrite (D) that is contacted (arrow) by an unlabeled terminal (uT). (C) GFP-ir is found in a terminal, identified by the presence of small synaptic vesicles (ssv; example), that forms a synapse (arrow) on an unlabeled dendrite. (D) In rare instances, GFP-ir is detected in astrocytes. The nuclei of these two astrocytes contain heterochromatin (hc). The astrocyte on the right also contains GFP reaction product (green arrows) in the cytoplasm. Note that the astrocytic processes conform to the boundaries of the neuropil. (A) PVN; (B, C) NTS; (D) RVLM. Bar A = 2 μm; B and C = 500 nm; D = 1 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Mice were anesthetized with CO$_2$, decapitated, and their brains rapidly removed and immersed in sucrose-artificial cerebrospinal fluid (aCSF). Coronal slices (300 μm in thickness) through the hypothalamus containing the PVN were cut on a vibratome (Leica VT100S) and stored in a custom-designed chamber containing lactic acid-artificial cerebrospinal fluid (l-aCSF) gassed with 95% O$_2$ and 5% CO$_2$ at 35 °C for 1 h. EGFP labeling was confirmed using a FITC filter on a Nikon EP6000 microscope. The PVN region then was removed using a micropunch, subjected to mild enzymatic digestion with 0.02% pronase and 0.02% thermolysin for 2 h and then stirred in the l-aCSF (Wang et al., 2006a,b). The dissociated PVN neurons were immediately moved to a 35 mm glass-bottom Petri dish.

ROS production was assessed using dihydroethidium (DHE) as an indicator (Kazama et al., 2004; Wang et al., 2008). For this, isolated neurons were incubated in DHE (2 μmol/L) for 30 min and then exposed throughout the measurement to DHE-containing buffer. The ROS measurement commenced when a stable baseline was achieved with vehicle (0.01% BSA in l-aCSF) and then AngII (100 nM; Sigma–Aldrich) was added to the buffer. Native EGFP in dissociated cells initially was detected using the FITC spectrum of Leica TCS SP5 confocal microscope. In all experiments, concurrent vehicle recording was performed until there was no difference in DHE fluorescence intensity before AngII application. Statistical significance was analyzed using one-way ANOVA. A $p$ value $<0.05$ was considered significant.

### RESULTS

#### Description of Agtr1a (AT1aR) BAC transgenic mouse line

To assess the fidelity of the Agtr1a BAC transgenic reporter mice, we first determined the cellular distribution of EGFP-containing cells in the brains of males and females to determine how it compares to that reported previously (Lenkei et al., 1997; Hauser et al., 1998; Daubert et al., 1999) for AT$_1$R binding, message and ir (albeit using different antisera than the present study).

#### Morphology of EGFP cells in the Agtr1a (AT1aR) BAC transgenic mouse brain

EGFP cells were examined in the brains of male and female Agtr1a BAC transgenic reporter mice identified either with native fluorescence or enhanced with immunocytochemistry. EGFP was detected using light microscopy throughout the nuclei and cytoplasm of individual and more densely packed cells (Fig. 2A–C). Most cells in Agtr1a BAC mice that were labeled with EGFP (i.e., AT1aR-EGFP) ranged in size from 10 to 20 μm. Single lightly labeled (Fig. 2A), single darkly labeled (Fig. 2B) or clusters of darkly labeled cells (Fig. 2C) were seen in regionally specific distributions. No GFP immunoreactive cells were seen in the brains of non-transgenic littermates that were processed simultaneously with the brains of Agtr1a BAC transgenic mice (Fig. 2A–C). The regional distributions and morphology of AT1aR-EGFP-containing cells appeared similar in male and female mice. Quantitative analysis revealed that the number of AT1aR-EGFP cells in male and female Agtr1a BAC transgenic mice in select brain regions was not significantly different (Table 1).

#### Ultrastructural morphology of AT1aR-EGFP cells

<table>
<thead>
<tr>
<th>GFP neurons</th>
<th>GFP glia</th>
<th>Percent neurons (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFO</td>
<td>103</td>
<td>0</td>
</tr>
<tr>
<td>PVN</td>
<td>104</td>
<td>1</td>
</tr>
<tr>
<td>RVLM</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>NTS</td>
<td>27</td>
<td>1</td>
</tr>
</tbody>
</table>

Total cells from 3 male Agtr1a BAC mice.

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**Fig. 4.** Effect of AngII on ROS production in isolated AT1aR-EGFP cells. (A) When viewed with fluorescence microscopy, native EGFP is visible in isolated cells. (B) In the presence of vehicle, DHE reaction product (pseudocolored red) is visible in the EGFP-containing cells shown in A. (C) Following the addition of AngII (100 nM), the intensity of DHE increases in the EGFP cells. Bar = 12 μm. (D) AngII increases ROS production in the EGFP cells, an effect blocked by the receptor antagonist losartan (Los; 3 mM). **$p < 0.01$ from vehicle; *$p < 0.05$ from AngII. n = 8 cells/group from three animals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Fig. 5. Distribution of AT1aR-EGFP cells in coronal sections of the Agtr1a BAC transgenic mouse brain. Drawings are arranged from rostral (A) to caudal (L) and are separated by a distance of 500–800 μm. The drawings are modified from Hof et al. (2000) and represent a composite qualitative distribution of the brains from 3 male and 4 female Agtr1a BAC transgenic mice. Light cells = open circle symbol (○); dark cells = solid circle symbol (●); clusters of cells = the asterisk symbol (*). In select regions, the number of AT1aR-EGFP cells was quantified (see Table 1). Bar = 1 mm. Abbreviations used in the figures: AAA, anterior amygdala area; ACBc and ACBs, nucleus accumbens core and shell; AC, anterior cingulate cortex; ac, anterior commissure; AHN, anterior hypothalamic nucleus; AO, anterior olfactory nucleus; AP, area postrema; ARH, arcuate nucleus of the hypothalamus; AUD, auditory cortex; Avp, anteroventral preoptic nucleus; AVPV, anteroventral periventricular nucleus of the hypothalamus; B, Barrington nucleus; BLA, basolateral amygdala; BLP, BLA posterior part; BMA, basomedial nucleus of the amygdala;
ultrastructurally in four brain regions known to be important in autonomic regulation (Allen et al., 2000). Almost all AT1R-EGFP cells were neurons in the four brain regions examined (Fig. 3 and Table 2). Specifically, GFP-labeled cells: (1) had a nucleus with a nucleolus; (2) had abundant cytoplasm with Golgi, endoplasmic reticulum and mitochondria and lacked fibrils; and (3) had dendrites that were contacted by terminals (Fig. 3A, B). Additionally, GFP-labeling was found in terminals (Fig. 3C). However, an occasional GFP-labeled glial cell was seen in the PVN, NTS and RVLM (Fig. 3D and Table 2). In all cases, the glial cell was classified as an astrocyte.

Analysis of isolated EGFP cells in the Agtr1a (AT1aR) BAC transgenic mouse brain

To determine if Agtr1a BAC transgenic mouse brains were suitable for in vitro studies, we examined ROS production (Wang et al., 2006b) in isolated EGFP-labeled cells. We chose the PVN of the hypothalamus to sample since this region has an abundance of AT1R-containing cells (Lenkei et al., 1997; Hauser et al., 1998). Prominent EGFP labeling in 300-μm slices though the PVN was seen prior to dissociating the cells (not shown). We found that isolated EGFP-containing cells from the PVN of male Agtr1a BAC transgenic mice maintained intense native EGFP labeling (Fig. 4A). In the presence of vehicle, DHE fluorescence was clearly distinguishable from native EGFP (Fig. 4B). Following application of AngII (100 nM), ROS production increased in EGFP-containing cells (Fig. 4C, D). This increase was blocked by preapplication of LOS (3 mM) (Fig. 4D). These findings are consistent with previous studies (Zimmerman et al., 2004; Wang et al., 2008) and demonstrate the utility of the AT1aR BAC transgenic mice in cell and molecular signaling studies.

Distribution of EGFP cells in the Agtr1a BAC transgenic mouse brain

We next determined the distribution of GFP-containing cells in Agtr1a BAC transgenic mouse brains to compare this distribution with that shown previously for AT1R binding, protein and mRNA in the brains of rats and mice (Rowe et al., 1990; Lenkei et al., 1997; Hauser et al., 1998). For the anatomical studies presented below, EGFP was localized in Agtr1a BAC transgenic mice using immunoperoxidase methods to increase the signal and cellular resolution of EGFP as well as yield a permanent reaction product that was visible using the light microscope. The distribution pattern of GFP cells was identical in females and males. The cell distributions presented in the maps in Fig. 5, are a composite made from examining the brains of 4 female (two in diestrus and one estrus) and 3 male Agtr1a BAC transgenic mice. The maps present a qualitative comparison of the number of cells in different brain regions.

Forebrain. AT1aR-EGFP cells were found in the tenia tecta, particularly in the ventral region (Fig. 5A). A few scattered AT1aR-EGFP cells were found near the lateral olfactory tract (Fig. 5A), the caudatus and the endopiriform and piniform cortices (Fig. 4B–D).

The basal forebrain nuclei contained numerous AT1aR-EGFP cells. A significant number of AT1aR-EGFP cells were found in the entire rostrocaudal extent of the dorsal and intermediate portions of the lateral septal nuclei (Fig. 5B, C). Some AT1aR-EGFP cells also were distributed throughout the medial septum and diagonal band of Broca (Fig. 5B). AT1aR-EGFP cells were found scattered throughout the nucleus accumbens (Fig. 5B), all subdivisions of the bed nucleus of the stria terminalis (Fig. 5C) and substantia
innominata (Fig. 5C). Intense AT1aR-EGFP labeling also was seen in the median preoptic area (Fig. 5C) and in the organum vasculosum of the lamina terminalis (OVLT; not shown).

Dense clusters of AT1aR-EGFP cells were seen in the caudate-putamen, particularly in the dorsolateral aspects (Figs. 5B, C and 6C). Numerous, intensely labeled AT1aR-EGFP cells were seen in both the dorsolateral outer shell and ventromedial core of the SFO (Figs. 5D and 6A, B). Consistent with AT1R mRNA and binding studies (Allen et al., 2000), dense GFP labeling also was found in the choroid plexus below the fimbria/fornix (fx) (Fig. 6A).

Thalamus, amygdala and hypothalamus. Only a few regions in the thalamus contained AT1aR-EGFP cells. In the rostral regions of the thalamus, scattered lightly labeled AT1aR-EGFP cells were found in the lateral habenula, reticular thalamic nucleus (Fig. 5D) as well as the parafascicular nucleus of the thalamus and neighboring areas (Fig. 5E). More caudally, intergeniculate leaflet (Fig. 5F) and the ventral subregion of the medial geniculate nucleus (Fig. 5G) contained small clusters of AT1aR-EGFP cells. In addition, a few scattered AT1-EGFP cells were observed in the substantia nigra, especially the pars reticulata (Fig. 5F, G).

AT1R-EGFP cells were detected in many of the nuclei within the amygdala complex. Numerous AT1aR-EGFP cells were in the basolateral (Fig. 5D) and central nuclei, especially the medial and central parts of the central nucleus (Figs. 4D and 5D). A few scattered AT1aR-EGFP cells were found in the anterior amygdaloid area and basal medial nucleus of the amygdala (Fig. 5D) as well as the posterior lateral and medial cortical nuclei of the amygdala (Fig. 5E, F).

In the hypothalamus, AT1aR-EGFP cells were densely packed in the PVN (Figs. 5D and 7A). To elucidate the location of GFP cells within PVN subregions, sections through the PVN were dually labeled with AVP, a marker of magnocellular neurons (Coleman et al., 2009). Most AT1aR-EGFP cells were

Fig. 6. Distribution of AT1aR-EGFP labeling in select forebrain regions. (A) Numerous AT1aR-EGFP cells are found in the SFO. Moreover, dense labeling for AT1aR-EGFP is found in the choroid plexus (cp) located below the fornix (fx). (B) At higher magnification, many dark AT1aR-EGFP cells were found in both the core and shell (sh) of the SFO. (C) Numerous clusters of AT1aR-EGFP cells (examples black chevrons) are found in the caudate-putamen (CP), especially in the dorsolateral quadrant. cc, corpus callosum. (D) Dispersed light and dark AT1aR-EGFP cells were seen in both the central and medial regions of part of the central nucleus of the amygdala (CEAc and CEAm, respectively). Bar A and D = 100 μm; B = 50 μm; C = 500 μm.
intermixed, but not colocalized, with AVP-labeled neurons suggesting that most AT1aR-EGFP cells are parvicellular neurons (Fig. 7B–D). Several AT1aR-EGFP cells were detected in the arcuate nucleus, dorsomedial and ventromedial hypothalamic nuclei and the periventricular nucleus of the hypothalamus (Fig. 5D) and interfascicular nucleus (Fig. 5G). Intensely labeled AT1aR-EGFP processes were seen in the median eminence (not shown). Lightly labeled AT1aR-EGFP cells were found in the suprachiasmatic nucleus (not shown), the caudal supraoptic nucleus and dorsal region of the periaqueductal gray area (Fig. 5H). A few scattered AT1aR-EGFP cells were observed in the medial preoptic area, the anterior hypothalamic nucleus, the lateral hypothalamic area (Fig. 5D, C) and the medial and lateral tuberal nucleus (not shown). The medial regions of the supramammillary and mammillary nuclei contained several AT1aR-EGFP cells (Fig. 5F).

**Cerebral and cerebellar cortices and hippocampus.** A significant number of lightly labeled AT1aR-EGFP cells were found throughout the rostrocaudal extent of all subdivisions of the cerebral cortex. Most of the AT1aR-EGFP cells were located in layers 5 and 6; some cells were scattered throughout the other layers (Fig. 5A–H). In the motor cortex, the AT1aR-EGFP cells tended to be organized in columns (Fig. 5B–D). Many small AT1aR-EGFP cells were detected in the principal cell layers of the anterior cingulate and retrosplenial cortices, especially the ventral aspects (Fig. 5B, E).

AT1aR-EGFP cells were dispersed throughout the hippocampal formation, however, the density of labeled cells varied with subregion. Dorsally, scattered lightly labeled AT1aR-EGFP cells were found in the stratum radiatum of CA1 and CA3 and in the molecular layer and hilus of the dentate gyrus (Fig. 5E–G). Ventrally, dense clusters of more intensely labeled AT1aR-EGFP cells were seen in the hilus of the dentate gyrus (Fig. 5F, G). Based on location and morphology, the few AT1aR-EGFP cells observed in the hippocampus were likely interneurons (Freund and Buzsáki, 1996). AT1aR-EGFP cells were sparsely distributed in layers 5 and 6 of the entorhinal cortex in a manner similar to that described above for the cerebral cortex.

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**Fig. 7.** Distribution of AT1aR-EGFP cells in the PVN of the hypothalamus. (A) Numerous dark AT1aR-EGFP-containing cells are found throughout the PVN. With confocal microscopy, cells containing AT1aR-EGFP (B) and AVP-ir (C) overlap (merged image D), but are distinct populations, in the PVN. Bar A–D = 50 μm.
In the cerebellum, a few lightly labeled AT1aR-EGFP cells were detected in the simple lobule (Fig. 5I) and the central lobe (Fig. 5J).

**Pons and medulla.** Within the pons, a few lightly labeled AT1aR-EGFP cells were detected in the rostrocaudal extent of the raphe nuclei, especially the dorsal subgroup (Fig. 5H). Scattered AT1aR-EGFP cells were located in the principal sensory nucleus of the trigeminal nerve (Fig. 5H), the midline region of the periaqueductal gray and gigantocellular reticular nucleus (Fig. 5I). More caudally, numerous AT1aR-EGFP cells were detected in all subregions of the lateral and medial parabrachial nucleus (PBN) and the locus coeruleus (LC) (Figs. 5H, I and 8A). Dual labeling studies demonstrated that almost all AT1aR-EGFP cells in the LC contained TH-ir (Fig. 8B–D). Several AT1aR-EGFP cells were noted in the Kölliker-fuse nucleus (KF), the dorsal tegmental nucleus (DTN), the ventral portion of the principal sensory nucleus of the trigeminal nerve, the gigantocellular reticular nucleus, the lateral paragigantocellular reticular nucleus and intermediate reticular nucleus (Fig. 5I).

In the medulla, lightly labeled AT1aR-EGFP-containing cells continued to be found in the raphe nuclei, especially the magnus and pallidus subdivisions (Fig. 5I, J). Numerous AT1aR-EGFP cells were detected in the NTS, especially in the medial and commissural subdivisions (Figs. 5J, K and 9A). Several AT1aR-EGFP immunoreactive processes and a few lightly labeled cells also were seen in the AP (Fig. 5K). Within the RVLM, many dark AT1aR-EGFP cells were detected (Figs. 5J and 10A). Consistent with our previous immunocytochemical studies (Glass et al., 2005; Wang et al., 2008; Pierce et al., 2009), additional dual label confocal studies revealed that almost all AT1aR-EGFP cells contained TH-ir in the NTS (Fig. 9B–D) and RVLM (Fig. 10B–D). Scattered dark and light AT1-EGFP cells were found in the region spanning between the NTS and RVLM; this region includes the nucleus prepositus, intermediate and medullary reticular nucleus and external cuneate nucleus (Fig. 5J, K). Several AT1aR-EGFP cells were present in both the interpolar and caudal parts of the spinal nucleus of the trigeminal nerve, especially medial to the spinal trigeminal tract (Fig. 5J).

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**Fig. 8.** Distribution of AT1aR-EGFP in the dorsal pons. (A) At low magnification, numerous dark AT1aR-EGFP cells are found in the locus coeruleus (LC), lateral and medial parabrachial nucleus (lPBN and mPBN, respectively) and interspersed in the Kölliker-fuse nucleus (KF) and the lateral dorsal tegmental nucleus (LDT). With confocal microscopy, AT1aR-EGFP (B) and TH-ir (C) are colocalized (merged image D) in the locus coeruleus. Bar A = 300 μm; B–D = 50 μm.
EGFP cells in the Agtr1a BAC reporter mouse contain AT1R-ir

Next, we confirmed the fidelity of EGFP in the Agtr1a BAC transgenic mouse by electron microscopic analysis of sections of tissue processed for dual labeling of GFP using silver-intensified gold (SIG) and AT1R using immunoperoxidase. The dual labeled somata had the morphology of neurons (Peters et al., 1991) in all brain regions examined, which included the PVN, central nucleus of the amygdala, and medullary nuclei involved in autonomic regulation.

SFO. Numerous perikarya and dendrites contained immunoreactivities for both AT1aR-EGFP and AT1R (Fig. 11A–C). AT1aR-EGFP SIG particles were distributed throughout the cytoplasm and nuclei, whereas AT1R-ir was found exclusively within the cytoplasm where it was sometimes affiliated with mitochondria or the plasma membrane (Fig. 11B).

PVN. Several perikarya and dendrites also contained SIG for AT1aR-EGFP in the PVN. Unlike the SFO, the majority of processes dually labeled for AT1aR-EGFP and AT1R-ir were dendrites in this brain region (Fig. 12A, B). Like the SFO, AT1R immunoperoxidase labeling was found in patches within the cytoplasm where it was sometimes affiliated with the plasma membrane (Fig. 12A) and endoplasmic reticulum (Fig. 12B).

Central nucleus of the amygdala. AT1aR-EGFP and AT1R-ir were colocalized in numerous perikarya and dendritic processes within the central nucleus of the amygdala (Fig. 13A, B). As in other brain regions, AT1aR-EGFP SIG particles were seen in the nucleus and cytoplasm whereas the AT1R-ir was partitioned to the cytoplasm of perikarya and dendrites in the central amygdala. In the cytoplasm, AT1R-ir was seen often in clusters.

Medulla. AT1aR-EGFP and AT1R-ir were found occasionally in a single axon terminal contacting and unlabeled dendrite or dendritic spines in the AP, a brainstem circumventricular organ having similarity to the

Fig. 9. Distribution of AT1aR-EGFP cells in the area postrema and NTS. (A) Numerous AT1aR-EGFP cells are detected in the medial NTS (mNTS). Some light AT1aR-EGFP cells also are detected in the area postrema (AP). Cc, central canal; DMX, dorsal motor nucleus of vagus; ts, tractus solitarius. With confocal microscopy, AT1aR-EGFP (B) and TH-ir (C) are colocalized (merged image D) in the mNTS. Bar A = 100 μm; B–D = 25 μm.
SFO in having direct access to circulating AngII (Lenkei et al., 1997) (Fig. 14A). In these axon terminals the AT$_{1R}$-ir was often affiliated with the plasma membrane.

In the NTS and RVLM, AT$_{1aR}$-EGFP SIG particles were found throughout the nucleus and cytoplasm of perikarya and dendrites; some of these dendrites contained ir for AT$_{1R}$ (Fig. 14B, C). AT$_{1R}$-ir in dendrites was found in clusters throughout the cytoplasm (Fig. 14C). Thus, the subcellular distribution of AT$_{1aR}$-EGFP and AT$_{1R}$ in these regions are similar to those in other brain regions including the circumventricular organs.

**DISCUSSION**

The present study provides evidence supporting the fidelity of the Agtr1a BAC transgenic reporter mice. EGFP-containing cells in these mice are predominantly neurons that have a topographic distribution throughout the brain that is similar to that reported previously for AT$_{1R}$ mRNA, protein and binding. Dual label electron microscopic studies demonstrate that AT$_{1R}$-ir colocalizes with AT$_{1aR}$-EGFP in neurons in several brain regions. Furthermore, assessment of AngII-induced free radical production in isolated EGFP cells demonstrated feasibility of studies investigating AT$_{1aR}$ signaling ex vivo. The versatility and greater sensitivity of EGFP for detecting AT$_{1R}$-containing cells in the Agtr1a BAC transgenic mouse will provide a useful tool for future anatomical, physiological and molecular experiments.

**Methodological considerations**

These studies support the feasibility of using Agtr1a BAC transgenic mice in anatomical as well as in vitro physiological studies. First, in 300-μm slices and isolated cell preparations, native EGFP fluorescence was visible at the light level in soma and proximal processes allowing for easy identification. Moreover, the green signal of native EGFP was clearly distinguished from the DHE reaction product (pseudo colored red) that changed in intensity after application of AngII in the presence or absence of the LOS. Thus, these observations support the utility of the Agtr1a BAC mouse for patch-clamp physiological experiments as well as ROS measurements in isolated cell preparations. Second, consistent with previous studies of GFP in other brain regions (Bulloch et al., 2008; Justice et al., 2008; Sierra et al., 2008; Lazarenko et al., 2009; Milner et al., 2010), immunoperoxidase reaction

![Fig. 10. Distribution of AT$_{1aR}$-EGFP cells in the RVLM. (A) Several AT$_{1aR}$-EGFP cells are found in the RVLM. With confocal microscopy, AT$_{1aR}$-EGFP (B) and TH-ir (C) are colocalized (merged image D) in the RVLM. Bar A = 25 μm; B–D = 25 μm.]
product for GFP was found throughout the dendritic processes when viewed by electron microscopy. Moreover, AT1aR-EGFP SIG labeling for GFP was clearly distinguished from the peroxidase reaction product for AT1R in single sections prepared for electron microscopy. These findings support the technical

Fig. 11. Electron microscopic localization of AT1aR-EGFP and AT1R-ir in the SFO. (A) At low magnification, AT1aR-EGFP SIG particles (black dots; example green arrow) are found dispersed throughout the nucleus (N) and cytoplasm of a neuronal perikaryon whereas diffuse patches of AT1R-ir appear interspersed in the cytoplasm (examples magenta arrowheads). (B) In a dendritic profile, AT1aR-EGFP SIG particles (example green arrow) are found throughout the cytoplasm whereas patches of AT1R-ir are associated with the mitochondria (m; top magenta arrowhead) or plasma membrane (bottom magenta arrowhead). (C) At high magnification, diffuse peroxidase reaction product for AT1R-ir is found throughout the AT1aR-EGFP somata (black dots; example magenta arrow), er, endoplasmic reticula. Bar = 500 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
the feasibility of dual label ultrastructural studies, particularly those that make quantitative comparisons between groups of particular receptors (e.g., NMDA) or signaling molecules in EGFP-containing cells. Third, GFP immunoreaction product can be detected in paraformaldehyde-fixed tissue with or without acrolein and in the presence or absence of detergent allowing AT1aR-EGFP cells to be identified under a greater variety of experimental conditions. Fourth, AT1aR-EGFP can be immunolabeled with a chicken or rabbit anti-GFP. This substantially increases the versatility of dual (and even triple) light and electron microscopic studies since the GFP antiserum can be combined with antisera against many other antigens produced in a variety of species to be subsequently identified with peroxidase, fluorescence, or immunogold labels.

**Morphology of AT1aR-EGFP cells**

In agreement with recent studies (Chen et al., 2012), nearly all of the cells containing AT1aR-EGFP labeling had the morphology of neurons when viewed by light and electron microscopy. Moreover, the lack of detectable GFP labeling in GFAP-containing astrocytes and the strong colocalization of GFP with TH in the LC, RVLM and NTS further demonstrate that AT1aR-EGFP is almost exclusively in neurons. However, our high-resolution electron microscopic studies revealed AT1aR-EGFP rarely labeled astrocytes in some brain regions. These findings are in agreement with previous light microscopic studies showing a lack of colocalization of AT1R mRNA with GFAP (Lenkei et al., 1997) and our previous electron microscopic studies showing low-levels of AT1R-ir (using the same antibody used in the present study) in glial processes in the rat NTS and RVLM (Huang et al., 2003; Glass et al., 2005; Pierce et al., 2009). Moreover, they also support previous in vitro findings that the expression of AT1Rs in astrocytes and microglia is negligible under basal conditions (Wosik et al., 2007; Miyoshi et al., 2008; O’Callaghan et al., 2011). However, several studies have shown that AT1R expression is variable depending on experimental conditions. In rat primary astrocyte cultures, growth hormone can increase AT1R levels (Wyse and Sernia, 1997). Low levels of AT1R mRNA are detected in astrocytes from both Wistar–Kyoto and spontaneously hypertensive rats in basal conditions (Zelezna et al., 1992). Moreover, overexpression of AT1aRs in the RVLM using Ad transgene increases glial expression of AT1aR that in turn can increase blood pressure possibly by modulating endogenous AT1Rs in presympathetic neurons (Allen et al., 2006). Whether or not experimental conditions like changes in the hormonal milieu or hypertension alter the numbers of glial cells containing AT1aR-EGFP is a subject of future investigations.

The detection of AT1aR-EGFP primarily in neurons also could be due to species and/or methodological differences. Our previous electron microscopic studies localizing AT1R-ir in NTS and RVLM were performed in rats (Huang et al., 2003; Glass et al., 2005; Pierce et al., 2009); thus, it is possible that more glia in rats contain AT1-R compared to mouse. Moreover, the AT1-R antibody used in these studies was raised against a peptide from the rat AT1aR (Huang et al., 2003). Since the rat sequence of the AT1a-R peptide chosen to make the antibody differs from the mouse by one amino acid and has another amino acid difference after the final cysteine (FEVE versus SEVE), this could have affected antigenicity.

AT1aR-EGFP also was found in the choroid plexus, a region enriched in microvasculature (Scala et al., 2011). Although we did not examine the choroid plexus ultrastructurally, these findings support previous studies showing that AT1-R-ir is located in endothelial cells in the...
The distribution of AT1aR-EGFP is consistent with immunocytochemical, in situ hybridization and binding studies

The present study found the distribution of cells containing EGFP in the Agtr1a BAC transgenic mouse to be consistent with previous reports from immunocytochemistry, in situ hybridization and AngII binding studies (Rowe et al., 1990; Tsutsumi and Saavedra, 1991; Aldred et al., 1993; Lenkei et al., 1997; Hauser et al., 1998; Allen et al., 2000), with some minor variations. Our electron microscopic studies showing AT1R-ir in EGFP-containing cells in many brain regions further support the fidelity of the Agtr1a BAC transgenic mice. The topographic distribution of AT1aR-EGFP-containing cells was similar in males and females, although with a larger sample size it is possible that some differences in the number of cells (e.g., the LC) could attain statistical significance. Since EGFP labeling does not provide information as to the subcellular localization of AT1Rs, these findings do not contradict our previous electron microscopic studies (Wang et al., 2008; Pierce et al., 2009) showing sex differences in the levels and subcellular distribution levels of AT1-R in TH-containing dendrites in the RVLM.

In both female and male Agtr1a BAC transgenic mice more AT1aR-EGFP cells were found in the cerebral cortex and hippocampal formation, compared to previous immunocytochemical, in situ hybridization, and binding studies in mice and rats (Rowe et al., 1990; Lenkei et al., 1997; Hauser et al., 1998). However, the pattern of distribution within these regions was relatively consistent with previous reports (Lenkei et al., 1995b, 1997; Allen et al., 2000). This suggests that differences in the frequency of labeled cells might reflect differences in the relative sensitivities of the methods. In particular, since the Agtr1a BAC transgenic reporter mouse is created by inserting an EGFP cassette directly upstream of the coding sequence of AT1aR, EGFP may be more able to identify cells that contain low amounts of mRNA and/or protein (Gong et al., 2003). Alternatively, differences in the frequency of labeled AT1aR, EGFP cells might reflect differences in the pattern of immunostaining seen with various AT1R antibodies.

In agreement with studies in both mice and rats (Lenkei et al., 1997; Hauser et al., 1998; Allen et al., 2000), AT1aR-EGFP cells were noticeably absent in many of the thalamic and pontine nuclei. Consistent with studies in C57BL/6J mice (Hauser et al., 1998; Daubert et al., 1999), we found numerous AT1aR-EGFP cells in the caudate-putamen. Interestingly, these findings contrast with previous studies in rats (Rowe et al., 1990; Gehlert et al., 1991; Tsutsumi and Saavedra, 1991; Lenkei et al., 1995a) suggesting that there are some species differences.

Functional implications

The topographical pattern of AT1aR-EGFP cells observed in this study directly reflects the well-established distribution of AT1-R-sensitive cells involved in circuits regulating both fluid homeostasis and cardiovascular function. Notably, high numbers of AT1aR-EGFP cells were found in some circumventricular organs, such as the SFO and the OVLT, and more moderate numbers in others, such as the median eminence and AP. These structures, which lack a blood–brain barrier, can sense and respond to circulating levels of AngII.

AT1aR-EGFP cells also are concentrated in regions involved in regulating cardiovascular reflexes, and autonomic function. A number of AT1aR-EGFP cells were found in the PVN which is involved in the integration of neural control of sympathetic outflow (Pyner and Coote, 1998; Sawchenko et al., 1996; de Wardener, 2001; Benarroch, 2005). AT1aR-EGFP cells
were found in the PBN, NTS and the RVLM, all of which are involved in cardiovascular regulation (Aicher et al., 2000; Hayward, 2007; Bourassa et al., 2009).

AT1aR-EGFP-containing cells also were not restricted to areas relating to cardiovascular and fluid control. In particular, AT1aR-EGFP cells were present in the hippocampal formation, the amygdala, septal nuclei and limbic cortices, such as entorhinal and piriform cortex that are important in learning, memory and anxiety. Studies have reported that AngII, acting through AT1Rs, can inhibit long-term potentiation in both the hippocampal formation (Wayner et al., 1993) and amygdala (von Bohlen und and Albrecht, 1998). AT1aR-EGFP cells also were concentrated in the bed nucleus of the stria terminalis, the preoptic area, the raphe, LC and, as discussed, the PVN. AT1-R-containing cells in these regions, particularly the PVN and LC, are involved in sympathetic and hormonal response to stress (Dumont et al., 1999; Saavedra et al., 2011). AT1aR-EGFP cells also were found in areas that modulate thermoregulation (e.g., the lateral parabrachial, medial preoptic area, raphe pallidus) (Morrison and Nakamura, 2011).

CONCLUSION

AT1aR-EGFP-containing cells can be detected by both light and electron microscopy as well as in isolated cells prepared for physiological and molecular studies. Thus, the availability of Agt1aR BAC transgenic reporter mice presents a further opportunity to understand the involvement of cells containing AT1aRs in autonomic function as well as other important processes.

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