Direct evidence of intracrine angiotensin II signaling in neurons

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Submitted 8 May 2013; accepted in final form 2 January 2014

The renin-angiotensin system (RAS) exists not only as a circulating hormonal system regulating blood pressure and water/electrolyte homeostasis, but also as a local system, with important physiological/pathophysiological implications in several organs, including the brain (32, 47). In addition, increasing evidence supports the existence of an intracellular RAS, converging to intracellular synthesis and action of ANG II (14, 29). Whether systemic, local, or intracellular, the central effector peptide of RAS is ANG II, formed by sequential cleavage of angiotensinogen and intraneuronal generation of ANG II. How-ever, the function and signaling mechanisms of intraneuronal ANG II remains elusive. Since ANG II type 1 receptor (AT1R) is the major type of receptor mediating the effects of ANG II, we used intracellular microinjection and concurrent Ca2+ elevation to investigate the functionality of intracellular AT1R in neurons. We show that intracellular administration of ANG II produces a dose-dependent elevation of cytosolic Ca2+ concentration ([Ca2+]i) in hypothalamic neurons that is sensitive to AT1R antagonism. Endolysosomal, but not Golgi apparatus, disruption prevents the effect of microinjected ANG II on [Ca2+]i. Additionally, the ANG II-induced Ca2+ response is dependent on motorpathy and sensitivity to inhibition of PLC or antagonism of inositol 1,4,5-trisphosphate receptors. Furthermore, intracellular application of ANG II produces AT1R-mediated depolarization of hypothalamic neurons, which is dependent on [Ca2+]i, increase, and on cation influx via transient receptor potential canonical channels. In summary, we provide evidence that intracellular ANG II activates endolysosomal AT1Rs in hypothalamic neurons. Our results point to the functionality of a novel intraneuronal angiotensinergic pathway, extending the current understanding of intracrine ANG II signaling.

calcium; endoplasmic reticulum; renin-angiotensin system
manufacturer's protocol (OriGene Technologies, Rockville, MD). Cells were used 24–48 h after transfection.

**Neuronal cell culture.** Hypothalamic neurons were dissociated from neonatal (1- to 2-day-old) Sprague-Dawley rats (Ace Animal, Boyertown, PA) of both sexes, as previously described (7). Newborn rats were decapitated, and brains were quickly removed surgically and immersed in ice-cold Hanks' balanced salt solution (HBSS; Mediatech). The hypothalamus was identified, removed, minced, and subjected to enzymatic digestion (papain, 5 min, 37°C) and mechanical trituration in the presence of total medium [Neurobasal-A (Invitrogen, Carlsbad, CA) containing 1% GlutaMAX (Invitrogen), 2% penicillin-streptomycin-amphotericin B solution (Mediatech), and 10% fetal bovine serum]. Cells were cultured on round 25-mm glass coverslips coated with poly-L-lysine (Sigma-Aldrich) in six-well plates. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO2. The mitotic inhibitor cytosine β-arabinofuranoside (1 μM; Sigma-Aldrich) was added to the culture on day 3 to inhibit glial cell proliferation.

**Immunocytochemistry and confocal imaging studies.** At 48 h after hypothalamic neurons were transiently transfected with DsRed-tagged rat AT1R and green fluorescent protein (GFP)-Rab7 (Addgene, Cambridge MA) using TurboFectin 8.0 according to the manufacturer’s protocol, they were fixed with 4% paraformaldehyde, washed in PBS, and mounted with Dapi-Fluoromont-G (Southern Biotech, Birmingham, AL). Cells were imaged using a two-photon confocal microscope (model 710, Carl Zeiss) with a 100X oil-immersion objective and 1 digital zoom, with excitations set for 4',6-diamidino-2-phenylindole, GFP, and DsRed at 405, 488, and 561 nm, respectively. Images were analyzed using ZEN 2010 (Zeiss), as previously reported (28, 49).

**Ca2⁺ imaging.** Intracellular Ca2⁺ concentration ([Ca2⁺]i) was measured as previously described (7, 9, 16, 17, 49). Cells were

### Table 1. Control experiments

<table>
<thead>
<tr>
<th></th>
<th>Untransfected U2OS cells</th>
<th>DsRed-transfected U2OS cells</th>
<th>AT1R-transfected U2OS cells</th>
<th>Primary hypothalamic neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG II (10⁻⁸ M)</td>
<td>17 ± 6</td>
<td>28 ± 5</td>
<td></td>
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<tr>
<td>Control buffer</td>
<td>24 ± 4</td>
<td>22 ± 6</td>
<td></td>
<td></td>
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<tr>
<td>CV-11947 (10⁻⁷ M)</td>
<td>25 ± 4</td>
<td>57 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-123319 (10⁻⁷ M)</td>
<td>23 ± 5</td>
<td>32 ± 4</td>
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Values are means ± SE. Changes in intracellular Ca²⁺ concentration (Δ[Ca²⁺]) produced by ANG II, CV-11947, and PD-123319 in untransfected U2OS cells, DsRed-transfected U2OS cells, ANG II type 1 receptor (AT1R)-transfected U2OS cells, or hypothalamic neurons were negligible.

Fig. 1. Microinjection of ANG II elevates cytosolic Ca²⁺ concentration ([Ca²⁺]i) in U2OS cells expressing ANG II type 1 receptor (AT1R). A: averaged traces (n = 6) of Ca²⁺ responses to ANG II (10⁻⁹–10⁻⁷ M), ANG II + the AT1R antagonist CV-11947 (10⁻⁷ M), and ANG II + the AT1R antagonist PD-123319 (10⁻⁷ M). Arrows indicate time of injection. B: increases in [Ca²⁺], produced by 10⁻⁸–10⁻⁷ M ANG II in the absence and presence of AT1R or AT1R antagonist, *P < 0.00001 vs. control injections. #P < 0.00001 vs. 10⁻⁸ M ANG II. C–F: representative images showing AT1R-DsRed fluorescence and fura 2-AM ratio of fluorescence (F340/F380) before, during, and 6 min after microinjection of control buffer, ANG II (10⁻⁸ M), ANG II + CV-11947, or ANG II + PD-123319. Arrows indicate injected cells. Fluorescence scale (0–3) is shown in C.
Ca<sup>2+</sup>

Arrows indicate injected cells. Fluorescence scale (0–3) is shown in microinjected ANG II (10<sup>−9</sup> M) membrane potential (Eppendorf, Nikon). The ratio of the fluorescence signal at 340 nm to that at 380 nm was converted to Ca<sup>2+</sup> concentration (24). In Ca<sup>2+</sup>-free experiments, CaCl<sub>2</sub> was omitted.

**Intracellular microinjection.** Intracellular microinjections were performed using FemtotipsII, InjectMan NI 2, and FemtoJet systems (Eppendorf), as described elsewhere (9, 16, 17, 49). Pipettes were backfilled with an intracellular solution containing (in mM) 110 KCl, 10 NaCl, and 20 HEPES (pH 7.2) or the compounds to be tested. Pipettes were perfused with an intracellular solution containing 0.5 mM DiBAC<sub>4</sub>(3), and fluorescence was monitored at 0.17 Hz and 480-nm excitation/540-nm emission. Calibration of DiBAC<sub>4</sub>(3) fluorescence following background subtraction was performed using the Na<sup>+</sup>-K<sup>+</sup> ionophore gramicidin in Na<sup>+</sup>-free physiological solution and various concentrations of K<sup>+</sup> (to alter V<sub>m</sub>) and N-methylglucamine (to maintain osmolarity). Under these conditions, V<sub>m</sub> was approximately equal to the K<sup>+</sup> equilibrium potential determined by the Nernst equation. The intracellular K<sup>+</sup> and Na<sup>+</sup> concentrations were assumed to be 130 and 10 mM, respectively.

**Measurement of membrane potential.** The relative changes in membrane potential (V<sub>m</sub>) of single neurons were evaluated using bis-1,3-dibutylbarbituric acidtrimethine oxonol [DiBAC<sub>4</sub>(3)], a slow-response voltage-sensitive dye, as previously described (49). Upon membrane hyperpolarization, the dye concentrates in the cell membrane, leading to a decrease in fluorescence intensity, while depolarization induces sequestration of the dye into the cytosol, resulting in an increase in the fluorescence intensity. Cultured hypothalamic neurons were incubated for 30 min in HBSS containing 0.5 mM DiBAC<sub>4</sub>(3), and fluorescence was monitored at 0.17 Hz and 480-nm excitation/540-nm emission. Calibration of DiBAC<sub>4</sub>(3) fluorescence following background subtraction was performed using the Na<sup>+</sup>-K<sup>+</sup> ionophore gramicidin in Na<sup>+</sup>-free physiological solution and various concentrations of K<sup>+</sup> (to alter V<sub>m</sub>) and N-methylglucamine (to maintain osmolarity). Under these conditions, V<sub>m</sub> was approximately equal to the K<sup>+</sup> equilibrium potential determined by the Nernst equation. The intracellular K<sup>+</sup> and Na<sup>+</sup> concentrations were assumed to be 130 and 10 mM, respectively.

**Data analysis.** Values are means ± SE. One-way ANOVA followed by post hoc Bonferroni’s and Tukey’s tests was used to assess significant differences between groups. P < 0.05 was considered statistically significant.

**RESULTS**

Similar to previous reports (51), to ensure that the effects of microinjected ANG II are not mediated by cell-surface receptors, in all series of experiments, plasma membrane AT<sub>1</sub>Rs and AT<sub>2</sub>Rs were blocked by bath application of CV-11947 and PD-123319 (both 10<sup>−7</sup> M), respectively.

**Control experiments.** Microinjection of control buffer or ANG II (10<sup>−8</sup> M) produced small, insignificant increases in
Calcium transient,[Ca^{2+}]i in untransfected U2OS cells. Similarly, in DsRed-transfected U2OS cells, intracellular administration of control buffer or ANG II had no effect. Also, microinjection of the AT1R antagonist CV-11947 or the AT2R antagonist PD-123319 (both 10^-7 M) did not elicit significant [Ca^{2+}]i increases in AT1R-transfected U2OS cells or neurons. Six cells were injected in each of the above-mentioned control experiments. Data are presented in Table 1.

**Intracellular injection of ANG II increases [Ca^{2+}]i in AT1R-transfected cells.** Microinjection of control buffer produced a small and insignificant [Ca^{2+}]i elevation of 27 ± 5 nM in AT1R-transfected U2OS cells, whereas intracellular administration of ANG II (10^-9, 10^-8, and 10^-7 M final intracellular concentrations) dose dependently increased [Ca^{2+}]i, by 258 ± 11, 711 ± 8, and 972 ± 17 nM, respectively (n = 6 cells for each concentration; Fig. 1, A–D). The rise in [Ca^{2+}]i was abolished when ANG II (10^-8 M) was coinjected with the AT1R antagonist CV-11947 (10^-7 M, Δ[Ca^{2+}]i = 18 ± 5 nM, n = 6) but was basically unaltered upon coadministration of the AT2R antagonist PD-123319 (10^-7 M, Δ[Ca^{2+}]i = 697 ± 7 nM, n = 6; Fig. 1, A, B, E, and F). Neither antagonist alone had an effect on its own when injected into AT1R-transfected U2OS cells (Table 1).

**Intracellular injection of ANG II activates AT1R in hypothalamic neurons.** Microinjection of control buffer did not significantly elevate [Ca^{2+}]i in cultured hypothalamic neurons (Δ[Ca^{2+}]i = 22 ± 4 nM, n = 19 neurons; Fig. 2, A–C), while intracellular administration of ANG II (10^-9, 10^-8, and 10^-7 M final intracellular concentrations) produced fast, transient, and dose-dependent increases in [Ca^{2+}]i: 197 ± 6 nM (n = 6 of 34), 398 ± 7 nM (n = 6 of 29), and 605 ± 9 nM (n = 6 of 32), respectively (Fig. 2, A–D). The percentages of neurons responding to increasing concentrations of ANG II by elevated [Ca^{2+}]i were 17.6%, 20.7%, and 18.75%, respectively, indicating that functional intracellular AT1R expression is restricted to a subpopulation of hypothalamic neurons.

**Treatment with CV-11947 (10^-7 M), an AT1R antagonist, but not PD-123319 (10^-7 M), an AT2R antagonist, prevented the Ca^{2+} response to microinjected ANG II (10^-8 M): Δ[Ca^{2+}]i = 17 ± 5 nM (n = 22 of 22 neurons) in the presence of CV-11947 and 391 ± 8 nM (n = 6 of 31) in the presence of PD-123319 (Fig. 2, A, B, E, and F). Pretreatment of neurons with the fast Ca^{2+} chelator BAPTA-AM (200 μM, 30 min) abolished the effect of ANG II (10^-8 M) microinjection (Δ[Ca^{2+}]i = 33 ± 5 nM, n = 63 of 63; Fig. 2, A and B).

**Endolysosomal location of functional intracellular AT1R in neurons.** Disruption of the Golgi apparatus by 1 h of incubation with brefeldin A (10 μM) (5) did not significantly affect the ANG II (10^-8 M)-induced Ca^{2+} response of hypothalamic neurons (Δ[Ca^{2+}]i = 411 ± 9 nM, n = 6 of 36 neurons; Fig. 3, A and B). Nevertheless, the ANG II effect was largely abolished by 1 h of incubation with bafilomycin A1 (1 μM), a V-type ATPase inhibitor that prevents lysosomal acidification (6), or rapamycin (30 μM), an agent that blocks microautophagy (30): Δ[Ca^{2+}]i = 28 ± 4 nM (n = 17 of 17) in neurons pretreated with bafilomycin A1 and 26 ± 6 nM (n = 33 of 33) in neurons pretreated with rapamycin (Fig. 3, A and B). Endolysosomal location of AT1R in neurons was confirmed by colocalization of AT1R-DsRed with Rab7-GFP, a small GTPase associated with endosomes and lysosomes (45) (Fig. 3C).

**Activation of endolysosomal AT1R in neurons mobilizes Ca^{2+} from inositol 1,4,5-trisphosphate-dependent stores.** In hypothalamic neurons incubated with Ca^{2+}-free HBSS, ANG II (10^-8 M) produced a robust increase in [Ca^{2+}]i, with an amplitude of 283 ± 9 nM (n = 6 of 35; Fig. 4A). This effect was slightly, but significantly, reduced compared with the amplitude of the same treatment in Ca^{2+}-containing saline (398 ± 7 nM, n = 6 of 29 neurons; Fig. 4A), indicating mobilization of extra- and intracellular Ca^{2+} pools by ANG II. The areas under the curve of the ANG II-induced responses in the presence and absence of extracellular Ca^{2+} in hypothe-
indicating that the increase in \( \text{Ca}^{2+} \) from intracellular and extracellular pools. A–C: amplitudes (A), areas under the curve (B), and half-lives (\( T_{1/2} \)) of \( \text{Ca}^{2+} \) responses to ANG II (10^{-9} \text{M}) in \( \text{Ca}^{2+} \)-containing and \( \text{Ca}^{2+} \)-free saline. *P < 0.00001 vs. \( \text{Ca}^{2+} \)-containing saline.

The ANG II-dependent \( \text{Ca}^{2+} \) response in \( \text{Ca}^{2+} \)-free saline was abolished in the presence of rapamycin (\( \Delta[\text{Ca}^{2+}]_i = 23 \pm 4 \text{nM}, n = 23 \)) or the PLC inhibitor U-73122 (\( \Delta[\text{Ca}^{2+}]_i = 24 \pm 5 \text{nM}, n = 26 \)).

Hypothalamic neurons depolarize upon stimulation of endolysosomal AT1R. Mean resting \( V_m \) in cultured hypothalamic neurons was \(-52.9 \pm 0.1 \text{ mV} (n = 173) \). Microinjection of control buffer did not modify the neuronal \( V_m \) (\( \Delta V_m = 0.1 \pm 0.1 \text{ mV}, n = 37 \)), whereas intracellular administration of ANG II (10^{-8} \text{ M}) depolarized 19% of hypothalamic neurons, with a mean amplitude of 3.74 ± 0.14 mV (n = 6 of 31; Fig. 6). When the AT1 antagonist CV-11947 (10^{-7} \text{ M}) was coadministered with ANG II, the effect of ANG II was abolished (\( \Delta V_m = 0.3 \pm 0.1 \text{ mV}, n = 34 \)). Conversely, coadministration of the AT1 antagonist PD-123319 (43) had no effect on the resting \( V_m \) of hypothalamic neurons.

Expression of several subtypes of TRPC channels has been documented in the human and rodent hypothalamus (19, 39). SKF-96365 (2 \text{ \mu M}) did not block the \( \text{Ca}^{2+} \) elevation in response to KCl (30 mM; Fig. 7), which produces depolarization-induced activation of voltage-gated \( \text{Ca}^{2+} \) channels; thus, at the concentration tested in this study, SKF-96365 is devoid of inhibitory activity at voltage-gated \( \text{Ca}^{2+} \) channels. diacylglycerol (DAG) has been reported to promote TRPC channel activation downstream of PLC (44), in the present study, the membrane-permeable DAG analog OAG (100 \text{ \mu M}) (43) had no effect on the resting \( V_m \) of hypothalamic neurons.
As such, functional intracellular AT₁ receptors in neurons is unlikely to be a mediator of ANG II-induced depolarization via TRPC channels.

**DISCUSSION**

While the presence of an intracellular RAS in neurons has been proposed for four decades (20), the functional significance of intraneuronal ANG II remained unclear. The effects and signaling pathways of ANG II in the brain have been extensively studied (3, 40, 46, 47). The contribution of brain ANG II to central control of blood pressure, drinking behavior, and salt appetite is indisputable (46, 47); these effects are mainly AT₁R-mediated (46, 47).

In light of increasing evidence of the intracrine role of ANG II (14, 17), we used concurrent Ca²⁺-imaging and microinjection techniques to assess the role of intracellular ANG II in hypothalamic neurons. Initial experiments in AT₁R-transfected U2OS cells confirm the validity of our methods and selectivity of receptor antagonists. Our results indicate that intracellular AT₁R is functional in hypothalamic neurons; microinjection of ANG II produced a dose-dependent increase in [Ca²⁺], mediated by AT₁R activation. Roughly 20% of neurons were ANG II-sensitive, indicating that the response may be selective to discrete neuronal populations in hypothalamic nuclei.

In an additional series of experiments, we examined the intracellular location of functional AT₁R in hypothalamic neurons. We found that disruption of lysosomes, but not the Golgi apparatus, completely prevented the effect of microinjected ANG II. In the absence of reliable anti-AT₁R antibodies (18, 26), we examined the distribution of AT₁R and the endolysosomal-associated small GTPase Rab7 (45) in hypothalamic neurons and identified extensive colocalization, supporting the endolysosomal location of AT₁R suggested by the functional studies.

Increasing evidence indicates that GPCRs may signal from the endolysosomal compartment (9, 16, 17, 27). This is not surprising, since the endolysosomal membranes are organized into specialized domains where molecules can assemble into specific signaling complexes (23, 41). However, the binding pocket for ANG II is situated on the NH₂-terminal side of the AT₁R and, thus, within the endolysosomal lumen, a site that is...
not immediately accessible for peptidic ligands. Nevertheless, we previously demonstrated that ANG II (17) or endothelin-1 (16) applied to the cell cytosol can be readily transferred inside the endolysosomal vesicles via microautophagy, a constitutive process by which soluble substrates are directly engulfed (35). A similar mechanism seemed plausible in the case of neuronal lysosomes. Indeed, rapamycin, an inhibitor of the last stage of the microautophagic process (30), prevented the effect of intracellular ANG II in hypothalamic neurons. Interestingly, the response was very fast, while the microautophagic process is expected to occur rather slowly. The time necessary for microautophagy is largely dependent on the lysosomal membrane invagination step of the process, which occurs with a 20- to 30-min lag; the subsequent vesicle scission step is very rapid, occurring in seconds (30, 35). Given that microautophagy is an ongoing process, important in housekeeping and in maintenance of cytoplasmic mass (35), membrane invaginations are formed continuously, and cytoplasmic components may be rapidly taken up into the endolysosomal lumen. The microautophagic vesicles, as generated, contain cytoplasmic medium, which is expected to preserve ANG II unmodified. After vesicle scission, hydrolases act to break down the vesicle and release microautophagic components into the lysosomal lumen, while permeases, such as Atg22p, promote recycling of nutrients and energy (35).

Next, we sought to characterize the signaling pathway triggered by activation of endolysosomal AT1R in hypothalamic neurons. Incubation of neurons in Ca\(^{2+}\)-free saline reduced the

![Diagram of ANG II-induced activation of endolysosomal AT1R in hypothalamic neurons](image.png)

**Fig. 8.** Proposed mechanism of ANG II-induced activation of endolysosomal AT1R in hypothalamic neurons. Cytosolic ANG II activates AT1R located on the endolysosomes (Endo-Lys) upon microautophagy-dependent transfer to the vesicle lumen. This, in turn, stimulates phospholipase C (PLC) at the lysosomal membrane to produce IP3 and subsequent endoplasmic reticulum (ER)/lysosomal Ca\(^{2+}\) release via IP3 receptors (IP3Rs). Release of Ca\(^{2+}\) from the ER triggers activation of transient receptor potential canonical (TRPC) channels at the plasma membrane and Na\(^+\) and Ca\(^{2+}\) influx, resulting in membrane depolarization.
amplitude and the area under the curve of the [Ca^{2+}]_{i} rise in response to intracellular ANG II, pointing to the occurrence of Ca^{2+} release and Ca^{2+} influx. IP_{3}R blockade or microautophagy inhibition, but not inhibition of nicotinic acid adenine dinucleotide phosphate-dependent endolysosomal Ca^{2+} release or ryanodine receptor blockade, significantly reduced the neuronal Ca^{2+} response to microinjected ANG II, indicating that the endoplasmic reticulum (ER)- or lysosome-located IP_{3}R (4, 21) are critically associated with endolysosomal AT_{1}R signaling. AT_{1}R couples to G_{q/11}-PLC to generate IP_{3} (15); this pathway is functional in hypothalamic neurons (42). In addition, PLC is expressed in the endolysosomal membrane (36); we found that PLC inactivation prevents the neuronal response to microinjected ANG II.

Since ANG II modulates hypothalamic function via depolarization and/or increase in excitability of target neurons (2, 10, 34), we further assessed whether intracellular administration of ANG II affects V_{m} in cultured hypothalamic neurons. A subpopulation of tested neurons depolarized in response to microinjected ANG II; the response was AT_{1}R-mediated and dependent on microautophagy. The percentage of neurons responding with a change in V_{m} correlated well with the proportion of neurons in which [Ca^{2+}]_{i} was elevated in response to ANG II microinjection. Whereas the mean amplitude of the depolarization triggered by microinjected ANG II is rather small (3.74 ± 0.14 mV), it is largely similar to that elicited by the bath-applied peptide in the hypothalamic neurons in the paraventricular nucleus that project to the rostral ventrolateral medulla (11). This angiotensinergic pathway is involved in blood pressure regulation (47), endorsing a physiological relevance of the change in V_{m} observed upon endolysosomal AT_{1}R activation. Changes in resting V_{m} determine the ease with which excitable synaptic inputs bring V_{m} closer to the threshold for action potential firing. A ~4-mV depolarization of hypothalamic neurons in the paraventricular nucleus was associated with a fourfold increase in the firing frequency of action potential discharge in response to ANG II (11). Since DiBAC_{4}(3) is a slow-response voltage-sensitive dye, fast changes in V_{m} and neuronal firing in response to ANG II microinjection may have been missed in the present study.

ANG II has been shown to depolarize hypothalamic neurons of supraoptic or paraventricular nuclei via activation of nonselective cation channels (31, 48). Since mobilization of Ca^{2+} from intracellular stores is a prerequisite for the activation of nonselective cationic conductance (38), this appeared to be a likely explanation linking the two main events triggered by intracellular ANG II in cultured hypothalamic neurons. Indeed, we noticed that chelation of intracellular Ca^{2+} with BAPTA-AM abolished the Ca^{2+} response and the depolarization elicited by ANG II. Furthermore, blocking Ca^{2+} entry via the TRPC channel, a nonselective cation channel activated via PLC by Ca^{2+} release from the ER (12, 44) or directly by DAG (44), also abolished the depolarization. In our experimental paradigm, however, DAG seemed not to be involved in TRPC channel activation, since OAG, a DAG analog used to study the existence of DAG-sensitive currents (43), could not mimic ANG II-induced depolarization in hypothalamic neurons.

Corroborating the findings of this study, we propose that, in hypothalamic neurons, intracellular ANG II may reach the AT_{1}R in the endolysosomes by microautophagy. ANG II stimulates endolysosomal AT_{1}R, leading to PLC activation and IP_{3}-dependent Ca^{2+} release. The release of Ca^{2+} from the ER, in turn, activates nonselective cation channels, such as TRPC channels, at the plasma membrane, promoting cation entry and neuronal membrane depolarization. The proposed model is summarized in Fig. 8. Considering the plethora of effects elicited by ANG II in the hypothalamus (46, 47), our findings increase the understanding of intracrine ANG II signaling in neurons with implications for neuronal physiology.

GRANTS
This work was supported by National Heart, Lung, and Blood Institute Grant HL-090804 (to E. Brailoiu).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


