Angiotensin II Type 1 Receptor–Mediated Inhibition of K⁺ Channel Subunit Kv2.2 in Brain Stem and Hypothalamic Neurons

Craig H. Gelband, John D. Warth, Helen S. Mason, Mingyan Zhu, Jennifer M. Moore, James L. Kenyon, Burton Horowitz, Colin Sumners

Abstract—Angiotensin II (Ang II) has powerful modulatory actions on cardiovascular function that are mediated by specific receptors located on neurons within the hypothalamus and brain stem. Incubation of neuronal cocultures of rat hypothalamus and brain stem with Ang II elicits an Ang II type 1 (AT₁) receptor–mediated inhibition of total outward K⁺ current that contributes to an increase in neuronal firing rate. However, the exact K⁺ conductance(s) that is inhibited by Ang II are not established. Pharmacological manipulation of total neuronal outward K⁺ current revealed a component of K⁺ current sensitive to quinine, tetraethylammonium, and 4-aminopyridine, with IC₅₀ values of 21.7 µmol/L, 1.49 mmol/L, and 890 µmol/L, respectively, and insensitive to α-dendrotoxin (100 to 500 nmol/L), charybdotoxin (100 to 500 nmol/L), and mast cell degranulating peptide (1 µmol/L). Collectively, these data suggest the presence of Kv2.2 and Kv3.1b. Biophysical examination of the quinine-sensitive neuronal K⁺ current demonstrated a macroscopic conductance with similar biophysical properties to those of Kv2.2 and Kv3.1b. Ang II (100 nmol/L), in the presence of the AT₂ receptor blocker PD123,319, elicited an inhibition of neuronal K⁺ current that was abolished by quinine (50 µmol/L). Reverse transcriptase–polymerase chain reaction analysis confirmed the presence of Kv2.2 and Kv3.1b mRNA in these neurons. However, Western blot analyses demonstrated that only Kv2.2 protein was present. Coexpression of Kv2.2 and the AT₁ receptor in Xenopus oocytes demonstrated an Ang II–induced inhibition of Kv2.2 current. Therefore, these data suggest that inhibition of Kv2.2 contributes to the AT₁ receptor–mediated reduction of neuronal K⁺ current and subsequently to the modulation of cardiovascular function. (Circ Res. 1999;84:352-359.)

Key Words: angiotensin II ■ Kv2.2 ■ Kv3.1b AT₁ receptor ■ Xenopus oocyte ■ cultured neuron

Mammalian brain contains specific angiotensin II (Ang II) type 1 (AT₁) receptors that are localized mainly in specific nuclei within the hypothalamus and brain stem. Some of these nuclei lie outside of the blood-brain barrier and sense circulating Ang II. Ang II acts at these receptors located on neurons to stimulate increases in blood pressure, arginine vasopressin (AVP) release, salt appetite, and drinking behavior, effects that participate in the modulatory role of this peptide on extracellular fluid volume and cardiovascular hemodynamics. All of these effects of Ang II are abolished by pretreatment of the animals with losartan, a selective AT₁ receptor antagonist. The exact mechanisms by which Ang II exerts its pressor effect have not been fully elucidated, but they appear to involve an increase in sympathetic vasomotor activity and/or an increase in vasopressin release. These actions of Ang II presumably involve the modulation of neuronal activity via changes in the underlying ionic currents and channels.

Data from our group and from other investigators have established that in various cell types, Ang II potentiates both high voltage activated and low voltage activated Ca²⁺ currents and inhibits several K⁺ current subtypes. In the brain, these effects could lead to an increase in neuronal excitability and may help to explain the increase in sympathetic vasomotor activity seen with intracerebroventricular injection of Ang II, which would result in stimulation of the cardiovascular system. Therefore, modulation of the effects of Ang II on neuronal Ca²⁺ and K⁺ currents may represent a potential site for therapeutic intervention in the treatment of a variety of cardiovascular disorders including hypertension. However, the specific ion channels that underlie the Ang II–induced alterations in neuronal K⁺ and Ca²⁺ currents are unknown.

In the present study, we have dissected, using biophysical and pharmacological methods, the total outward K⁺ current in hypothalamus and brain stem neuronal cocultures prepared...
from the newborn rat. Results from these studies revealed that a major component of neuronal K⁺ current was sensitive to low doses of quinine, tetraethylammonium (TEA), and 4-aminopyridine (4-AP) and insensitive to α-dendotoxin (α-DTX), charbybdotoxin, and mast cell degranulating peptide (MCDP), suggesting the presence of a “Kv2.2- or Kv3.1b-like” current.15,16 Ang II, which normally inhibits neuronal K⁺ current,11 had no significant effects on K⁺ current in the presence of quinine. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of the neuronal cocultures demonstrated the presence of mRNA for Kv2.2 and Kv3.1b. However, Western blot analyses showed that these cultures expressed Kv2.2 and not Kv3.1b protein. Coexpression of mRNA for Kv2.2 and the AT1α receptor in Xenopus oocytes resulted in an Ang II–dependent inhibition of Kv2.2 current. Therefore, these data suggest that inhibition of Kv2.2 contributes to the Ang II–induced reduction of neuronal outward K⁺ current.

Materials and Methods
Preparation of Neuronal Cultures
Neuronal cocultures were prepared from brain stem and a hypothalamic block of newborn Sprague-Dawley rats as previously described.17,18 Cultures were grown for 10 to 14 days. At this time, the cultures consisted of ~90% neurons and 10% astroglia.

Whole-Cell Patch Clamp of Cultured Neurons
Membrane currents in isolated neurons were measured using the whole-cell variation of the patch-clamp technique.19 Patch electrodes were pulled from glass capillary tubing (Corning 7052), fire-polished, and had resistances of 2 to 4 MΩ. Patch electrodes were pulled from glass capillary tubing (Kimax-51, Kimble Products) with resistance of 1 to 3 MΩ.

Materials and Methods
Preparation of Neuronal Cultures
Neuronal cocultures were prepared from brain stem and a hypothalamic block of newborn Sprague-Dawley rats as previously described.17,18 Cultures were grown for 10 to 14 days. At this time, the cultures consisted of ~90% neurons and 10% astroglia.

Whole-Cell Patch Clamp of Cultured Neurons
Membrane currents in isolated neurons were measured using the whole-cell variation of the patch-clamp technique.19 Patch electrodes were pulled from glass capillary tubing (Corning 7052), fire-polished, and had resistances of 2 to 4 MΩ. Patch electrodes were pulled from glass capillary tubing (Kimax-51, Kimble Products) with resistance of 1 to 3 MΩ.

Materials and Methods
Preparation of Neuronal Cultures
Neuronal cocultures were prepared from brain stem and a hypothalamic block of newborn Sprague-Dawley rats as previously described.17,18 Cultures were grown for 10 to 14 days. At this time, the cultures consisted of ~90% neurons and 10% astroglia.

Whole-Cell Patch Clamp of Cultured Neurons
Membrane currents in isolated neurons were measured using the whole-cell variation of the patch-clamp technique.19 Patch electrodes were pulled from glass capillary tubing (Corning 7052), fire-polished, and had resistances of 2 to 4 MΩ. Patch electrodes were pulled from glass capillary tubing (Kimax-51, Kimble Products) with resistance of 1 to 3 MΩ.

Materials and Methods
Preparation of Neuronal Cultures
Neuronal cocultures were prepared from brain stem and a hypothalamic block of newborn Sprague-Dawley rats as previously described.17,18 Cultures were grown for 10 to 14 days. At this time, the cultures consisted of ~90% neurons and 10% astroglia.

Whole-Cell Patch Clamp of Cultured Neurons
Membrane currents in isolated neurons were measured using the whole-cell variation of the patch-clamp technique.19 Patch electrodes were pulled from glass capillary tubing (Corning 7052), fire-polished, and had resistances of 2 to 4 MΩ. Patch electrodes were pulled from glass capillary tubing (Kimax-51, Kimble Products) with resistance of 1 to 3 MΩ.
After either incubation, the membrane was washed 1 time for 15 minutes and 4 times for 5 minutes in PBST at room temperature. Detection of the resulting antigen-antibody-peroxidase complex was done with use of the Renaissance (Du Pont–NEN) enhanced chemiluminescence (ECL) kit, according to the manufacturer’s directions, and visualized by exposure to Kodak film (BioMax Light) for 30 seconds.

Data Analysis
All results are expressed as mean±SEM and were obtained from combining data from individual experiments. Comparisons of multiple means were made using an analysis of variance, followed by a Newman-Keuls test to assess statistical significance. A value of P<0.05 was determined to be significant. Dose-response relationships were fit to the following equation: \( I/I_{\text{max}} = 1 + ([\text{drug}]/I_{\text{C50}})^{-1} \).

Results
As a first step toward elucidating the underlying K+ conductances that contribute to the outward current in these neuronal cocultures, we characterized basic biophysical properties of the total outward K+ current using the whole-cell, patch-clamp technique. Figure 1 shows the voltage dependence of activation (Figure 1A and 1B) and inactivation (Figure 1C and 1D) of the outward current. The activation curve was obtained from analysis of the peak amplitude of tail current elicited after depolarization of the membrane to potentials ranging from −80 to +40 mV for 500 ms followed by a step to −50 mV for 150 ms from a holding potential of −80 mV to elicit tail currents. Peak tail current was then plotted against the membrane potential of the test pulse and fit to a single Boltzmann function. The smooth line through the data points reveals a threshold for activation of approximately −20 mV, half-maximal activation at −0.23±1.88 mV (n=7), and a slope factor of 9.4±0.6 (n=7). The voltage-dependent inactivation of this current was obtained using 10-second conditioning pulses to various potentials followed by 2.5-second test steps to +20 mV. Inactivation during the conditioning pulse was not complete at the end of the 10-second pulse, and, therefore, only approximates the steady-state inactivation. The magnitude of the current was measured at the end of the voltage pulse to avoid any contamination of the A-type K+ current, which has been shown to inactivate with a time constant of approximately 14 ms. The data were normalized to the maximal current and then plotted as a function of the conditioning step potential. The resulting curve was then fit with a single Boltzmann function with half inactivation at −29.6±0.34 mV (n=5) and a slope factor of −8.4±0.9 (n=5).

Recovery from inactivation of the total outward current was also examined using a protocol in which neurons were held at −80 mV and then stepped for 10 seconds to +20 mV as a conditioning pulse, back to −80 mV for recovery of various duration, and then to +20 mV for 2-second test pulses. A plot of the magnitude of the current at the end of the test pulse versus the recovery interval shows that there are at least 2 phases to the recovery, including a relatively rapid initial phase during which 50% of the current recovers (15±3 ms, n=6) followed by a prolonged phase requiring more than 2 seconds for the current to fully recover (2123±23 ms, n=6). These 2 phases fit well with the recovery kinetics of inactivating and noninactivating Kv channel subtypes.

As an additional step in characterizing the outward K+ current in these neurons, various pharmacological agents were used. Figures 2A, 2C, and 2E show raw current traces obtained under control conditions and after various doses of TEA, 4-AP, or quinine, respectively. The current before and after increasing concentrations of blocker was measured at +20 mV (end of the pulse), and the data were plotted as the percentage of current remaining versus the concentration of blocker. The apparent IC50 values for block by these various agents were calculated as TEA 1.49±0.21 mmol/L (Figure 2B), 4-AP 0.89±0.10 mmol/L (Figure 2D), and quinine 21.7±2.66 μmol/L (Figure 2F). Kv gene products that may
underlie this current may be Kv1.1, Kv1.3, Kv2.2, and Kv3.1b. To further pharmacologically dissect the K⁺ current, charybdotoxin (100 to 500 nmol/L), α-DTX (100 to 500 nmol/L), and MCDP (1 μmol/L) were also tested, because these agents have been shown to inhibit Kv1.1 and Kv1.3. These agents were without any significant effect on total K⁺ current (data not shown).

It has recently been reported that both Kv2.2 and Kv3.1b display a unique sensitivity to low doses of quinine, suggesting that either Kv2.2, Kv3.1b, or a combination of these channels may underlie the quinine-sensitive current component, because other K⁺ channels show a sensitivity to quinine that is at least a log unit higher than that of Kv2.2. To test for this possibility, an IC₅₀ dose of quinine (25 μmol/L) was used to isolate the quinine-sensitive K⁺ current component. This dose was chosen because it significantly inhibits the outward K⁺ current in our neuronal cultures, but it should have minimal effects on other known K⁺ channels. Outward currents were recorded in the absence and presence of quinine using activation and inactivation protocols, and then the currents in the presence of quinine were subtracted from the control currents, allowing for the isolation of the quinine-sensitive K⁺ current.

**Figure 3.** Voltage-dependent activation and inactivation of the quinine-sensitive K⁺ current. A and C, Raw current traces for voltage-dependent activation and inactivation, respectively. Voltage-clamp currents were recorded using identical protocols to those outlined in Figure 1. The quinine-sensitive current was defined as the difference current obtained by subtracting the current in the presence of quinine (25 μmol/L) from the current obtained under control conditions. B and D, I-V relationships for the activation (n=4) and inactivation (n=3) of the quinine-sensitive current, respectively. Voltage-dependent activation was obtained by plotting the magnitude of the tail current, normalized to the maximum at +40 mV, versus the step potential. Voltage-dependent inactivation was plotted as the magnitude of the current at the end of the 2.5-second test pulse (normalized to the maximum test current obtained) and plotted as a function of the step potential of the conditioning pulse.
half inactivated at $-38.6\pm0.55$ mV ($n=3$) and has a slope factor of $-8.8\pm0.93$ ($n=3$). A comparison of the properties of Kv2.2, Kv3.1b, and the quinine-sensitive K$^+$ current is presented in the Table and demonstrates that these 3 conductances share several biophysical and pharmacological similarities.\textsuperscript{15,16,21–24}

We have previously reported that Ang II decreases delayed rectifier K$^+$ current in cultured neurons in a Gq/protein kinase C (PKC)/calcium/calmodulin protein (CAM) kinase II–dependent manner.\textsuperscript{11,25} Therefore, the effect of Ang II on the quinine-sensitive K$^+$ current was next examined. Figure 4 illustrates that Ang II (100 nmol/L), in the presence of the AT$_2$ receptor blocker PD123,319 (1 \(\mu\)mol/L), is capable of decreasing K$^+$ current, an effect that is reversible on washout. Quinine (50 \(\mu\)mol/L) also inhibits K$^+$ current but to a greater extent. In the presence of quinine, Ang II produced no significant reduction in K$^+$ current. These results suggest that the regulation of Kv current by Ang II is via a quinine-sensitive Kv channel(s).

To test for the possibility that Kv2.2 and/or Kv3.1b might be contributing to the outward current in these neurons, RT-PCR analysis was performed. Amplification of total RNA followed by separation of amplification products on agarose gels revealed products of the expected size for Kv2.2 (550 bp) and Kv3.1b (437 bp) when RT was included in the reaction (Figure 5A, lanes 3 and 9) but not when this enzyme was omitted (Figure 5A, lanes 2 and 8), suggesting the presence of Kv2.2 and Kv3.1b mRNA. Given that these neuronal cocultures are contaminated by a small population ($\sim10\%$) of astroglial cells, it was possible that the observed product was amplified from the glial cells and not from the neurons. This possibility was tested in 2 ways. Total RNA was isolated from neonatal rat astroglial cultures and subjected to RT-PCR with identical primers to those used in the neuronal cocultures. Amplification of RNA isolated from these pure astroglial cultures failed to produce a band for Kv2.2 or Kv3.1b when separated on an agarose gel, despite the fact that the RNA was intact as evidenced by the amplification of \(\beta\)-actin from the same RNA sample (data not shown). Because these astroglial cultures must be grown under different serum conditions than the neuronal cocultures, an argument could be made that this could be affecting the expression of Kv2.2 and Kv3.1b. To rule out this possibility, neuronal cocultures were treated with 100 nmol/L KCl for 10 minutes to kill the neurons. The KCl was washed out, and the remaining glial cells were allowed to grow under identical serum conditions as the neuronal cocultures for a period of 1 week. At this time,
there were no neurons present as determined by visual inspection. RNA was isolated from these cultured glial cells and subjected to RT-PCR. Again, no expression of Kv2.2 or Kv3.1b could be detected in the glial cultures (Figure 5A, lanes 6 and 12), although the RNA was intact as shown by amplification of lanes 5 and 11), although the RNA was intact as shown by inspection. RNA was isolated from these cultured glial cells and subjected to RT-PCR. Again, no expression of Kv2.2 or Kv3.1b. Lanes 1, 3, 8, and 10 are negative controls in which RT was omitted from the reaction mix using cultured neurons or brain regions but not in cultured neurons. Experiments were repeated at least 3 times.

We directly tested the possibility that Ang II is inhibiting Kv2.2 using the *Xenopus* oocyte expression system. The mRNAs for the AT1 receptor and for Kv2.2 (Figure 6) were injected into oocytes. One to 4 days after injection, a family of currents was elicited in the oocytes from a holding potential of −80 mV with steps to potentials ranging from −50 to +50 mV followed by repolarization to −40 mV. Application of Ang II (100 nmol/L) for 15 minutes resulted in a slowly developing inhibition of the outward current in oocytes coinjected with mRNA encoding Kv2.2 and the AT1 receptor. K+ currents were evoked by a voltage step from a holding potential of −80 mV to +50 mV followed by repolarization to −40 mV. A slowly developing inhibition of Kv2.2 can be seen after 15 minutes of exposure to Ang II, which continued to develop over a 10-minute wash period. B, Peak I-V relationship recorded from the oocytes in panel A. ○, control; ■, 15 minutes of Ang II; and ●, 10 minutes of wash. C, Time course of Ang II–induced inhibition of Kv2.2 in oocytes injected with either mRNA encoding Kv2.2 alone (○) or Kv2.2 plus the AT1 receptor (■). Kv2.2 currents were measured at a test potential of +50 mV and normalized to the magnitude of current measured under control conditions (n=6 to 9). Ang II was applied for 15 minutes as indicated, followed by a 30-minute wash. D, Pretreatment of 6 oocytes with the AT1 receptor antagonist losartan (1 μmol/L) prevented the Ang II–induced inhibition of Kv2.2 current. In 4 oocytes, reexposure to Ang II after washout of losartan inhibited the current.

Figure 6. AT1 receptor–mediated inhibition of Kv2.2 in *Xenopus* oocytes. A, Representative traces demonstrating inhibition of Kv2.2 current by Ang II (100 nmol/L) in oocytes coinjected with mRNA encoding Kv2.2 and the AT1 receptor. K+ currents were evoked by a voltage step from a holding potential of −80 mV to +50 mV followed by repolarization to −40 mV. A slowly developing inhibition of Kv2.2 can be seen after 15 minutes of exposure to Ang II, which continued to develop over a 10-minute wash period. B, Peak I-V relationship recorded from the oocytes in panel A. ○, control; ■, 15 minutes of Ang II; and ●, 10 minutes of wash. C, Time course of Ang II–induced inhibition of Kv2.2 in oocytes injected with either mRNA encoding Kv2.2 alone (○) or Kv2.2 plus the AT1 receptor (■). Kv2.2 currents were measured at a test potential of +50 mV and normalized to the magnitude of current measured under control conditions (n=6 to 9). Ang II was applied for 15 minutes as indicated, followed by a 30-minute wash. D, Pretreatment of 6 oocytes with the AT1 receptor antagonist losartan (1 μmol/L) prevented the Ang II–induced inhibition of Kv2.2 current. In 4 oocytes, reexposure to Ang II after washout of losartan inhibited the current.

The mRNAs for the AT1 receptor and for Kv2.2 (Figure 6) were injected into oocytes. One to 4 days after injection, a family of currents was elicited in the oocytes from a holding potential of −80 mV with steps to potentials ranging from −50 to +50 mV followed by repolarization to −40 mV. Application of Ang II (100 nmol/L) for 15 minutes resulted in a slowly developing inhibition of the outward current in oocytes coinjected with the AT1 receptor (0.73±0.03 of control at +50 mV, P<0.001; n=6) but not in oocytes injected only with Kv2.2 (0.99±0.02 of control at +50 mV, n=7) (Figure 6A through 6C). Pretreatment of the oocytes with losartan (1 μmol/L) prevented the Ang II–induced inhibition of Kv2.2 (0.93±0.02 of control at +50 mV, n=8), but after washout of losartan, a second application of Ang II decreased the outward current (0.67±0.09 of control at +50 mV, n=4) (Figure 6D). The time course of inhibition of Kv2.2 and lack of washout by Ang II were similar to inhibition of Kv1.5 and Kv1.2 by acetylcholine when coexpressed with the human M3 receptor in oocytes or COS cells.26 These experiments demonstrate that the inhibition of Kv2.2 is mediated through an AT1 receptor and not through the endogenous oocyte angiotensin receptor, because the endogenous receptor is not inhibited by losartan.27
Discussion

The existence of a local renin-angiotensin system in the brain is now well established.\(^1\) In addition, specific nuclei within the brain, collectively known as the circumventricular organs, lie outside the blood-brain barrier and possess AT\(_1\) receptors that sense circulating levels of Ang II.\(^2\) Centrally acting Ang II has been shown to induce release of AVP via a catecholaminergic pathway in the brain,\(^3\) increase sympathetic vasomotor activity,\(^4,5\) and therefore regulate a number of cardiovascularly relevant physiological events. Support for this idea comes from recent evidence demonstrating that AT\(_1\) and AT\(_2\) receptors are colocated on catecholaminergic neurons.\(^6\) Both the increase in sympathetic activity and release of AVP have been implicated as mediators of the vasopressor, elevated dipsogenic, and Na\(^+\) appetite responses and the increase in fluid volume observed with intracerebroventricular injection of Ang II. However, the underlying cellular mechanism for these responses is unclear.

Ang II, via AT\(_1\) receptors, results in an inhibition of neuronal K\(^+\) current in several cell types.\(^7,11,13\) This inhibition would depolarize or alter the refractoriness of the neuronal membrane, resulting in an increase in neuronal firing rate. Therefore, modulation of the specific K\(^+\) channel(s) inhibited by Ang II represents a potential therapeutic target site in the treatment of a variety of cardiovascular diseases including hypertension. However, the specific K\(^+\) channel(s) that is inhibited by Ang II has not been elucidated.

In the present study, we characterized the outward K\(^+\) current in neuronal cocultures of rat brain stem and hypothalamus in an attempt to identify the specific K\(^+\) conductance(s) modulated by Ang II. Electrophysiological experiments were used to define the biophysical properties of activation, inactivation, and recovery from inactivation for the total outward K\(^+\) current. However, the results from these studies are consistent with the properties of several different types of Kv channels.\(^15\) Pharmacological dissection of Kv current was then used to try and show which Kv channel subunit(s) was modulated by Ang II. It was first determined that a large portion (~80%) of total outward K\(^+\) current was sensitive to low doses of quinine (IC\(_{50}\) 21.7 \(\mu\)mol/L), TEA, and 4-AP. These data narrowed the candidate K\(^+\) channel subunits to Kv1.1, Kv1.3, Kv2.2, and Kv3.1b. Additional pharmacological analyses showed that total Kv current was insensitive to charybdotoxin, \(\alpha\)-DTX, and MCDP. Because Kv2.2 and Kv3.1b show a unique sensitivity to low doses of quinine when expressed in Xenopus oocytes,\(^15,16,23\) have similar sensitivities to TEA and 4-AP, and are relatively insensitive to charybdotoxin, \(\alpha\)-DTX, and MCDP, this suggested the presence of Kv2.2 and Kv3.1b in these neuronal cultures. Analysis of the quinine-sensitive K\(^+\) current revealed a conductance with similar biophysical properties to those reported for Kv2.2 and Kv3.1b, and in the presence of low doses of quinine, which is relatively specific for Kv2.2 and Kv3.1b, Ang II produced no inhibitory effect on neuronal K\(^+\) current. RT-PCR analyses confirmed the presence of Kv2.2 and Kv3.1b mRNA, lending additional support to the idea that Kv2.2 and/or Kv3.1b are components of total outward K\(^+\) current in these neurons. However, Western blot analyses demonstrated that of these 2 subunits, only Kv2.2 is present in the cultures.

The pharmacological and molecular data provided in the present study suggest that total outward K\(^+\) current is composed of a nonhomogeneous population of channels, because 20% to 30% of the current remained, despite the use of maximum concentrations of several inhibitors. Pharmacological dissection of total outward K\(^+\) current is made even more difficult by the fact that there are no specific inhibitors or blocking antibodies against Kv2.2, and, therefore, it was not possible to determine whether Ang II was solely inhibiting Kv2.2. Because the initial goal of the present study was to determine the specific K\(^+\) channel(s) present in these neurons that are inhibited by Ang II, we chose to use the Xenopus oocyte expression system to determine if Kv2.2 was inhibited by Ang II. The observed losartan-sensitive inhibition of Kv2.2 by Ang II suggests that these channels may be, at least partially, responsible for the observed Ang II–induced inhibition in cocultures of rat hypothalamus and brain stem. However, definitive in vitro proof of this observation will have to await the development of a specific inhibitor of Kv2.2.

Although the results from the present study have demonstrated the inhibition of Kv2.2 by Ang II, they have not ruled out the possibility that Ang II modulates other K\(^+\) channels. The present study also has not addressed the second messenger system through which this inhibition occurs. The AT\(_1\) receptor is traditionally believed to be coupled through a G-protein transduction mechanism involving the G\(_{i1}\)/G\(_{\beta\gamma}\) family. This family of G proteins typically activates phospholipase C-\(\beta\) (PLC-\(\beta\)), which catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate to inositol-1,4,5-trisphosphate (IP\(_3\)) and diacylglycerol (DAG). IP\(_3\) promotes Ca\(^2+\) release from the endoplasmic reticulum, and DAG activates PKC. Both of these pathways have been shown to be important in neuronal AT\(_1\) receptor signaling,\(^13\) but exactly how these mediators ultimately modulate neuronal K\(^+\) channels is unknown. Kv2.2 has a well-conserved PKC phosphorylation site between the S4 and S5 transmembrane segments.\(^15\) We have recently shown,\(^14\) using the oocyte expression system, that the inhibition of Kv2.2 by Ang II is blocked by PKC inhibitors and intracellular BAPTA, similar to that which was shown by Summers et al\(^13\) in hypothalamic/brain stem neurons in culture. Thus, it is possible that the Ang II–induced inhibition of Kv current is via a Ca\(^2+\)-dependent, PKC-mediated phosphorylation of Kv2.2. In neurons, there is also evidence that AT\(_1\) receptors couple to a stimulation of mitogen-activated protein (MAP) kinase.\(^35,36\) However, it is unclear if MAP kinases are also involved in the modulation of neuronal Kv current.

The discovery in the present study of a specific Kv channel inhibited by Ang II will allow for a more detailed examination of the intracellular signaling mechanism of the AT\(_1\) receptor in neurons that represent important targets for the regulation of cardiovascular events. It will also allow for a more direct assessment of whether cross-talk occurs between AT\(_1\) and AT\(_2\) receptors. The answers to these questions will be the focus of future research.

Acknowledgments

This work was supported by grants from the National Institutes of Health HL-49130 and NS-19441 (to C.S. and C.H.G.); DK-41315 (to...
References


Angiotensin II Type 1 Receptor–Mediated Inhibition of K⁺ Channel Subunit Kv2.2 in Brain Stem and Hypothalamic Neurons
Craig H. Gelband, John D. Warth, Helen S. Mason, Mingyan Zhu, Jennifer M. Moore, James L. Kenyon, Burton Horowitz and Colin Sumners

Circ Res. 1999;84:352-359
doi: 10.1161/01.RES.84.3.352

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/84/3/352

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/