A-Type K⁺ Current in Neurons Cultured From Neonatal Rat Hypothalamus and Brain Stem: Modulation by Angiotensin II

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A-type K⁺ current in neurons cultured from neonatal rat hypothalamus and brain stem: modulation by angiotensin II. I. Neurophysiol. 78: 1021–1029, 1997. The regulation of A-type K⁺ current (Iₐ) and the single channel underlying Iₐ in neonatal rat hypothalamus/brain stem cultured neurons were studied with the use of the patch-clamp technique. Iₐ had a threshold of activation between −30 and −25 mV (n = 14). Steady-state inactivation of Iₐ occurred between −80 and −70 mV and had a membrane voltage at which Iₐ was half-maximum at −52.2 mV (n = 14). The mean values for the activation and inactivation (decay) time constants during a voltage step to +20 mV were 2.1 ± 0.3 (SE) ms (n = 8) and 13.6 ± 1.9 ms (n = 8), respectively. Single-channel recordings from outside-out patches revealed A-type K⁺ channels with voltage-dependent activation, 4-aminopyridine (4-AP)-sensitivity, and inactivation kinetics similar to those of Iₐ. The single-channel conductance obtained from cell-attached patches was 15.8 ± 1.3 pS (n = 4) in a physiological K⁺ gradient and 41.2 ± 3.7 pS (n = 5) in symmetrical 140 mM K⁺. Angiotensin II (Ang II, 100 nM) reduced peak Iₐ by ~20% during a voltage step to +20 mV (n = 8). Similarly, Ang II (100 nM) markedly reduced single A-type K⁺ channel activity by decreasing open probability (n = 4). The actions of Ang II on Iₐ and single A-type K⁺ channels were reversible either by addition of the selective angiotensin type 1 (AT₁) receptor antagonist losartan (1 μM) or on washout of the peptide. Thus the activation of AT₁ receptors inhibits a tetraethylammonium-chloride-resistant, 4-AP-sensitive Iₐ and single A-type K⁺ channels, and this may underlie some of the actions of Ang II on electrical activity of the brain.

INTRODUCTION

In the nervous system, A-type K⁺ current (Iₐ) plays an important role in the control of excitability, synaptic input, and neurotransmitter release (Cull-Candy et al. 1989; Dye 1991; Mei et al. 1995; Sheng et al. 1993). Iₐ may be responsible for the initial repolarization of the action potential, contribute to afterhyperpolarization, promote pacemaker activity, and facilitate neuronal firing (Bouskila and Dudek 1995; Chandler et al. 1994; Connor and Stevens 1971; Dekin 1993; Duceux and Puiziott 1995; Grolleau and Lapied 1995; MacDermott and Weight 1982). Iₐ has been studied in a variety of mammalian nervous tissues including sympathetic ganglia (Galvan and Sedlmeier 1984), hypothalamus and hippocampal brain slices (Bouskila and Dudek 1995; Greene et al. 1990; Zbicz and Weight 1985), neonatal supraoptic nucleus neurons (Cobbett et al. 1989), and embryonic hypothalamus neurons (Muller et al. 1992). Electrophysiologically, Iₐ can be identified by its unique voltage-dependent activation, fast kinetics of inactivation, and steady-state inactivation. Pharmacologically, Iₐ is aminopyr-dine sensitive and has been shown to be modulated by acetylcholine, glutamate, adenosine, and γ-aminobutyric acid (Akins et al. 1990; Hay and Lindsley 1995; Mei et al. 1995; Saint et al. 1990). The cloned K⁺ channels from rat brain, RCK4 (Kv1.4) and Raw3 (Kv3.4), have been demonstrated to give rise to A-like currents (Pongs 1992; Schroter et al. 1991; Stuhmer et al. 1989).

Our previous work demonstrated that angiotensin II (Ang II), by activation of the angiotensin type 1 (AT₁) receptor subtype, caused a reversible and concentration-dependent delay in rectifier K⁺ current (Iₐ) in rat cultured neurons from hypothalamus and brain stem (Kang et al. 1992; Sumners et al. 1996). Using a brain slice preparation, Nagatomo et al. (1995) reported that Ang II decreased the Iₐ amplitude by 21.3 ± 3.1% (mean ± SE) in supraoptic neurons, whereas Li and Ferguson (1996) demonstrated that by activation of AT₁ receptors, Ang II reduced Iₐ by 31.0 ± 4.1% in paraventricular nucleus cells.

The single K⁺ channel that underlies Iₐ in cultured neurons has been characterized by several groups (Cooper and Shrier 1985; Kasai et al. 1986; Solé et al. 1987). Cooper and Shrier (1985) reported that in rat nodule sensory neurons A-type K⁺ channel conductance was ~22 pS and ensemble averaged single-channel currents had similar inactivation kinetics to that of Iₐ. Kasai et al. (1986) observed a 20-pS A-type K⁺ channel in guinea pig dorsal root ganglion cells. The conductance of single A-type K⁺ channels in mammalian cultured neurons was greater than that of Drosophila neurons (5–8 pS) (Solé et al. 1987) and of cloned A-type K⁺ channels (4.7–14 pS) (Stuhmer et al. 1989; Vega-Saenz de Miera et al. 1994). To date, Ang II regulation of the single channel underlying neuronal Iₐ has not been studied, although such modulation forms the basis for its action on whole cell currents and plays a role in modulating action potential firing patterns (see companion paper).

In this study, for the first time, we characterize a 4-aminopyridine (4-AP)-sensitive Iₐ and the single channel underlying it in rat cultured neurons with the use of the whole cell, outside-out, and cell-attached configurations of the patch-clamp technique. Furthermore, we demonstrate that the actions of Ang II on Iₐ and single A-type K⁺ channel currents are mediated through the AT₁ receptor.

METHODS

Materials

One-day-old Sprague-Dawley rats were obtained from our breeding colony, which originated from Charles River Farms (Wilmington, MA). Losartan potassium was generously provided by...
Dr. Ronald D. Smith of Du Pont-Merck (Wilmington, DE). PD 123319 (AT2 receptor blocker) was purchased from Research Biochemicals (Natwick, MA). Dulbecco’s modified Eagle’s medium (DMEM) was obtained from Gibco (Grand Island, NY). Crystalized trypsin (xl) was from Cooper Biomedical (Malvern, PA). Plasma-derived horse serum (PDHS), cytosine arabinoside (CytA), DNase I, poly-L-lysine (molecular weight 150,000), Ang II, ATP, guanosine 5'-triphosphate, tetraethylammonium chloride (TEA), 4-AP, tetrodotoxin (TTX), CdCl2, and N2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) were purchased from Sigma Chemical (St. Louis, MO). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

Preparation of neuronal cultures

Neuronal cocultures were prepared from the brain stem and a hypothalamic block of 1-day-old Sprague-Dawley rats as described previously (Kang et al. 1992). Trypsin (375 U/ml) and DNase I (496 U/ml) dissociated cells were resuspended in DMEM containing 10% PDHS and plated on poly-L-lysine precoated 35-mm Nunc plastic tissue culture dishes. After cells were grown for 3 days at 37°C in a humidified incubator with 95% air:5% CO2, they were exposed to 1 μM ARC for 2 days in fresh DMEM containing 10% PDHS. Then ARC was removed and the cells were incubated with DMEM (10% PDHS) for a further 9–12 days before use. At the time of use, cultures consisted of 90% neurons and 10% astrocyte glia, as determined by immunofluorescent staining with antibodies against neurofilament proteins and glial fibrillary acidic proteins (Sumners et al. 1990).

Electrophysiological recordings

Macroscopic and single-channel currents were recorded with the use of the whole cell, outside-out, and cell-attached patch-clamp configurations of the patch-clamp technique (Hamill et al. 1981). Experiments were performed at room temperature (22–23°C) with the use of an Axopatch-200A amplifier and Digidata 1200A interface (Axon Instruments, Burlingame, CA). Cells were bathed in Tyrode’s solution containing (in mM) 140 NaCl, 5.4 KCl, 2.0 CaCl2, 2.0 MgCl2, 0.3 NaH2PO4, 10 HEPES, and 10 dextrose, pH adjusted to 7.4 with NaOH. Neurons in the culture dish (volume 1.5 ml) were superfused at a rate of 2–4 ml/min. The patch electrodes (Kimax-5.1, Kimble Glass, Toledo, OH) had resistances of 3–4 MΩ when filled with an internal pipette solution containing (in mM) 140 KCl, 2 MgCl2, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 4 ATP, 0.1 guanosine 5'-triphosphate, 10 dextrose, and 10 HEPES, pH adjusted to 7.2 with KOH. Cell-attached patches were formed by sealing the patch electrode to the cell body. Single-channel activity was recorded from patches with a seal resistance >5 GΩ. The whole cell configuration was formed by applying negative pressure to the patch electrode. For whole cell recordings, cell capacitance was canceled electronically and the series resistance (<10 MΩ) was compensated by 75–80%. Outside-out patches were formed by slowly elevating the patch electrode away from the cell body after formation of the whole cell configuration. A junction potential of ~10 mV was corrected for all command potentials.

Sodium current (INa) was blocked by TTX (100 nM). Calcium current (ICa) was blocked by CdCl2 (100 μM). Ca2+-activated K+ current (IK(Ca)) was reduced to insignificant levels by omitting CaCl2 from the pipette solution, blocking ICa with CdCl2, and buffering intracellular free Ca2+ with EGTA. In most of the experiments, IK was blocked by extracellular TEA (140 mM), which replaced the NaCl (140 mM).

To evaluate the steady-state inactivation of IA, a depolarizing test pulse to +42.5 mV (200 ms in duration) was preceded by conditioning prepulses from −102.5 to −5 mV in 7.5-mV steps (1 s in duration). IA was measured as the peak current amplitude elicited by the depolarizing test pulses and expressed as the membrane conductance [GA = IA/(V − Vr)], where Vm is membrane potential and Vr is the K+ equilibrium potential (~82 mV) determined from the concentration gradient with the assumption that intracellular K+ was equal to pipette potassium (140 mM). In some cases, IA was converted to conductance values (nS).

In outside-out patch-clamp experiments, single A-type K+ channel activity was elicited with the use of pulses from −100 mV to various test potentials (0 to +40 mV). In cell-attached patch-clamp experiments, A-type K+ channel activity was elicited either with the use of voltage steps from +40 mV to various test potentials (~100 to 0 mV) when patch pipettes were filled with Tyrode’s solution (5.4 mM KCl) or by pulses from −100 mV to various test potentials (~20 to +100 mV) when patch pipettes were filled with 140 mM KCl. For convenience, the current–voltage relationship for single-channel currents obtained from cell-attached patches was constructed by plotting single-channel current amplitude as a function of the driving force (the pipette potential minus the average resting potential, ~60 mV when the bath contained normal Tyrode’s solution and −20 mV when the bath contained 140 mM TEA).

Data acquisition and analysis were performed with the use of pCLAMP 6.03. Whole cell currents were filtered at 1 kHz (frequency filter ~3 dB) and digitized at 2 kHz. Single-channel currents were filtered at 2 kHz (frequency filter −3 dB) and digitized at 10 kHz. By convention, outward current is depicted as upward deflections of current.

Data analysis

Results are expressed as means ± SE. Statistical significance was evaluated with the use of paired t-test. Differences were considered significant at P < 0.05; n corresponds to the number of cells examined. Open probability (NPo) for single A-type K+ channels was obtained during 100-ms depolarizing pulses.

RESULTS

Neurons used for the experiments had an oval- or triangular-shaped cell body with two or three small and short processes that did not form a network with adjacent cells. The maximum diameter of the neurons was ~15–25 μm. The passive membrane input resistance of the cells was 301.4 ± 21.7 MΩ (n = 45). The average cell capacitance was 51.15 ± 2.1 pF (n = 73). Typical neuronal action potentials, either spontaneous or stimulated, were present in these cells (see companion paper). There were at least four transmembrane currents that underlie the depolarization and repolarization of the action potential. These are defined as 1) TTX-sensitive INa, 2) Cd2+-sensitive ICa, 3) TEA-sensitive IK, and 4) 4-AP-sensitive transient K+ current (IA). The present work focuses on the 4-AP-sensitive IA.

Isolation of IA

In normal Tyrode’s solution, when INa and ICa were blocked by TTX (100 nM) and Cd2+ (100 μM), respectively, a voltage-dependent total outward current was recorded (Fig. 1A). The total outward current could be pharmacologically dissected into at least two components. When TEA (140 mM) was bath applied, significant inhibition of a delayed-rectifier-like K+ current was observed (Fig. 1B). Subtracting the current traces in Fig. 1B from those in Fig. 1A reveals an outward K+ current that is defined as a volt-
age-dependent, TEA-sensitive $I_K$ similar to that of the Kv class of $K^+$ channel (Fig. 1C). The second component of outward current was sensitive to 4-AP. Bath application of 4-AP (5 mM) significantly reduced the transient portion of the outward current (Fig. 1D). Subtracting the current traces in Fig. 1D from those in Fig. 1B reveals a voltage-dependent, transient outward $K^+$ current that was defined as a TEA-resistant and 4-AP-sensitive $I_A$ (Fig. 1E). $I_A$ activated rapidly and inactivated within 40 ms. The conductance-voltage relationship for $I_A$ is presented in Fig. 1F ($n = 14$).

**Biophysical properties of $I_A$**

Steady-state inactivation of $I_A$ was studied in a total of 14 neurons. The steady-state inactivation current traces of $I_A$ (Fig. 2) were obtained with the use of the voltage paradigms described in the METHODS section. The peak amplitude of $I_A$ elicited by repetitive depolarizing pulses (+42.5 mV) began to decrease at conditioning potentials of −80 to −70 mV (Fig. 3, A and C). When the conditioning potential was more positive than −65 mV, steady-state inactivation became accelerated and complete inactivation occurred at a conditioning potential of −27.5 mV. The averaged steady-state inactivation data were fit with the following Boltzmann function

$$\frac{g_A}{g_{A(max)}} = \frac{1}{1 + \exp[(V - V_{1/2})/k]}$$

where $g_A/g_{A(max)}$ is the conductance normalized to its maximum value, $V$ is the membrane potential, $V_{1/2}$ is the mem-

**Fig. 1.** Isolation of neuronal A-type $K^+$ current ($I_A$). Sodium current ($I_{Na}$) and calcium current ($I_{Ca}$) were blocked by tetrodotoxin (TTX, 100 nM) and Cd$^{2+}$ (100 μM), respectively. Cell membrane potential was held at −90 mV. A: total outward current traces elicited by 200-ms depolarizing pulse from −35 to +42.5 mV in 7.5-mV steps following 1-s prehyperpolarizing pulse to −110 mV. B: current traces recorded in presence of external tetraethylammonium chloride (TEA, 140 mM). C: TEA-sensitive difference current [$A − B$, mainly delayed rectifier $K^+$ current ($I_K$)]. D: current traces recorded in presence of TEA and 4-aminopyridine (4-AP, 5 mM). E: TEA-resistant, 4-AP-sensitive transient difference current ($I_A$) obtained by subtracting currents recorded in presence of 4-AP from currents recorded in absence of 4-AP (i.e., $B − D$). F: conductance-voltage relationship (current amplitude was measured as peak current during depolarizing pulse) of $I_A$. These results are representative of 14 cells.

**Fig. 2.** Voltage-dependent steady-state inactivation of $I_A$. Cell membrane potential was held at −90 mV. Experiments were performed in presence of TTX (100 nM), Cd$^{2+}$ (100 μM), and TEA (140 mM) in bath solution. A: representative current traces were elicited by 200-ms repetitive depolarizing pulses to +42.5 mV, which were preceded by 1-s conditioning prepulses from −102.5 to 5 mV in 7.5-mV steps in absence of 4-AP. B: current traces recorded in presence of 4-AP (5 mM). C: TEA-resistant, 4-AP-sensitive $I_A$ ($A − B$). D: steady-state inactivation curve of $I_A$ for 14 neurons. Points: normalized mean peak conductance of $I_A$ at each test potential. Solid line is obtained by fitting mean data with Boltzmann function.
brane voltage at which $I_A$ is half-maximum, and $k$ is the Boltzmann slope factor. The averaged data, when fit with a Boltzmann function, revealed a half-steady-state inactivation potential ($V_{1/2}$) of $-52.2$ mV and a voltage-sensitive slope factor ($k$) of $-6.5$ mV.

The activation and inactivation (decay during a voltage step) time constants of the 4-AP-sensitive $I_A$ were studied in eight neurons. Figure 3A shows a representative trace of $I_A$ during a 200-ms depolarizing voltage step from $-110$ to $+42.5$ mV. The activation (rising) and the inactivation (decaying) phases of the current were fit best by a single-exponential equation

$$I = I_{\text{max}}[1 - \exp(-t/\tau)]$$

where $I$ is the amplitude of $I_A$ at time $t$, $I_{\text{max}}$ is the peak $I_A$ at time 0, and $\tau$ is the time constant for activation or inactivation. In this experiment, activation and decay time constants were 1.4 and 14.3 ms, respectively. The mean values of activation and inactivation time constants for a depolarizing pulse to $+20$ mV were $2.1 \pm 0.3$ ms and $13.6 \pm 1.9$ ms, respectively.

**FIG. 3.** Activation and inactivation time constants of $I_A$. A: representative $I_A$ trace (——) elicited by depolarizing pulse from $-110$ to $+42.5$ mV. Dashed lines are obtained by fitting rising (activation) and decay phases (inactivation) of current trace with 1st-order exponential equation. $\tau_{\text{act}} = 1.4$ msec, $\tau_{\text{decay}} = 14.3$ msec.

The time to reach peak activation of $I_A$ was voltage dependent (Fig. 3B). The greater the depolarization, the faster $I_A$ activated. For example, $\tau$ was equal to $4.0 \pm 0.6$ ms during a depolarizing pulse to $+5$ mV but was only $1.1 \pm 0.1$ ms during a voltage step to $+42.5$ mV. In comparison, the inactivation (decay) time constant of $I_A$ was voltage dependent only when the depolarizing potential was more negative than 0 mV. When the depolarizing test potential was more positive than 0 mV, the decay time constant showed little voltage dependence (Fig. 3C).

**FIG. 4.** A-type K$^+$ channel activity in physiological K$^+$ gradient obtained from cell-attached patches. A: single-channel currents recorded from both cell-attached and outside-out patches. In physiological external K$^+$ solution [$K^+$ concentration in the pipette ($[K^+]_{\text{pip}}$) = extracellular K$^+$ concentration ($[K^+]_o$) = 5.4 mM]. Experiments were performed in presence of TTX (100 nM), Cd$^{2+}$ (100 $\mu$M), and TEA (140 mM) in bath solution. Currents were activated by depolarizations to different potentials (as indicated), which were preceded by 1-s hyperpolarizing pulses to $-100$ mV. B: current-voltage relationship from 4 patches. Chord conductance was measured by linear regression through data. $\gamma$, single-channel conductance.

**Single-channel characteristics of 4-AP-sensitive $I_A$**

Single neuronal A-type K$^+$ channel currents were recorded from both cell-attached and outside-out patches. In a physiological external K$^+$ solution [$K^+$ concentration in the pipette ($[K^+]_{\text{pip}}$) = extracellular K$^+$ concentration ($[K^+]_o$) = 5.4 mM], A-type K$^+$ channel activity occurred...
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Figure 5. Voltage-dependent activation and 4-AP sensitivity of single A-type K⁺ channels in outside-out patches. Patches were bathed in solution containing TTX (100 nM), Cd²⁺ (100 μM), and TEA (140 mM) and held at potential of −60 mV. A: ensemble average currents of single A-type K⁺ channels activated by depolarizing step from −100 to 42.5 mV. This is representative of 5 cells. B: single-channel activities in an outside-out patch, which was elicited by voltage step to +20 mV in absence (left) and presence (right) of 4-AP (5 mM). Recordings were from same cell. This is representative of 5 cells.

when the patch of membrane was depolarized to a potential more positive than −40 mV (Fig. 4A). Under these conditions, the single-channel conductance was 15.8 ± 1.3 pS (n = 4) and the extrapolated reversal potential was −85.2 ± 1.7 mV (n = 4, Fig. 4). This is close to the predicted equilibrium potential of K⁺ calculated with the use of the Nernst equation. Figure 5 illustrates biophysical and pharmacological fingerprints of single A-type K⁺ channel currents recorded from outside-out patches. During a voltage step from −100 to +40 mV, rapidly activating and inactivating channels were observed. The current trace illustrated in Fig. 5A is an ensemble average of 30 traces. The inactivation time constant of the ensemble averaged single-channel currents in this experiment was 16.7 ms and the average was 16.1 ± 4.2 ms (n = 4), which was similar to the decay time constant of Iₐ at +42.5 mV (15.3 ± 3.4 ms, Fig. 3). To pharmacologically fingerprint these inactivating channels, 4-AP (5 mM) was added to the bathing solution. 4-AP significantly decreased the activity of A-type K⁺ channels without affecting the single-channel current amplitude (Fig. 5B). On the basis of the above biophysical and pharmacological data, it is concluded that the activity of these 4-AP-sensitive, rapidly activating and inactivating single K⁺ channels underlies Iₐ.

Ang II regulation of Iₐ

We next examined the pharmacological regulation of Iₐ by Ang II in the presence of the AT₂ receptor antagonist PD 123319 (1 μM). PD 123319 had no effect on baseline K⁺ current or channel activity. Ang II, when superfused into the recording chamber, significantly reduced Iₐ in a dose-dependent manner (Fig. 6, A and B; asterisk: P < 0.01, n = 12 for Ang II (100 nM), n = 5 for the other concentrations). Therefore we chose to use a maximum effective dose of Ang II, 100 nM, in the rest of our experiments. Ang II (100 nM) reduced peak Iₐ and shifted the activation curve to the right in eight neurons tested (Fig. 7). Figure 7A shows a

Figure 6. Dose-dependent effect of angiotensin II (Ang II) on Iₐ. A: Iₐ recorded from neuron in presence of TTX (100 nM), Cd²⁺ (100 μM), and TEA (140 mM) in Tyrode’s solution. Traces 1–4: control and superfusion with 1, 10, and 100 nM Ang II, respectively. B: graph showing mean data for Ang II-mediated, dose-dependent decrease in peak Iₐ from 5 neurons. Asterisk: P < 0.01.
were reversed either on washout (Fig. 8, right) or by addition of losartan (1 μM) to the superfusate solution (data not shown). The ensemble averaged current is illustrated below the single-channel current traces and reflects control, application of Ang II, and washout, respectively. The ensemble averaged data are markedly similar to those data for Ang II regulation of \( I_A \) illustrated in Fig. 7. Similar results were observed in four other cell-attached patches. Figure 9 illustrates the effects of Ang II on single-channel \( N_{Po} \). The data in Fig. 9 were used to generate amplitude histograms and calculate \( N_{Po} \). Under control conditions, in the presence of Ang II (100 nM), and after washout, \( N_{Po} \) was 0.21, 0.10, and 0.16, respectively. The mean values for \( N_{Po} \) under these conditions were 0.22 ± 0.04, 0.10 ± 0.08, and 0.17 ± 0.11, respectively (\( n = 4 \)).

DISCUSSION

The transient outward \( K^+ \) current reported here was defined as a TEA-resistant, 4-AP-sensitive A current (\( I_A \)). This conclusion was based on the following criteria. First, under our experimental conditions, \( I_{Na}, I_{Ca}, I_K \), and \( I_{K, Ca} \) were blocked by TTX, Cd²⁺, TEA, and internal EGTA, (5 mM) respectively. Second, the voltage dependence and the kinetics of activation and inactivation of \( I_A \) were similar to previously published results (Figs. 1–3) (Bouskila and Dudek 1995; Mei et al. 1995). Finally, the single-channel conductance demonstrated for channels that underlie \( I_A \) was similar to that reported for other neuronal preparations (Figs. 4 and 5) (Cooper and Shrier 1985; Kasai et al. 1986).

This study also demonstrated that Ang II, via activation of \( \text{AT}_1 \) receptors, reduced \( I_A \) and decreased the activity of a 15-pS, rapidly inactivating, 4-AP-sensitive \( K^+ \) channel (Figs. 8 and 9). Our previous studies showed that hypothalamus and brain stem neuronal cocultures contained ~70% \( \text{AT}_2 \) and ~30% \( \text{AT}_1 \) receptors (Sumners and Raizada 1993; Sumners et al. 1991, 1994). We previously determined that activation of \( \text{AT}_1 \) receptors by Ang II decreases net outward current via inhibiting \( I_K \) and stimulating \( I_{Ca} \), which was dependent on protein kinase C activation (Sumners et al. 1996). Our recent study also showed that intracellular injection of a 25-amino-acid peptide corresponding to the third intracellular loop of the cloned \( \text{AT}_{1a} \) receptor (\( \text{AT}_{1a,c} \)) elicited changes in \( I_K \) and \( I_{Ca} \) that were similar to those obtained with application of Ang II via \( \text{AT}_1 \) receptors. By contrast, injection of a 19-amino-acid peptide corresponding to the second intracellular loop did not modulate \( I_K \) or \( I_{Ca} \). Importantly, our data elucidated that the modulation of neuronal \( I_K \) and \( I_{Ca} \) by \( \text{AT}_{1a,c} \) involves protein kinase C, inositol-(1,4,5)-trisphosphate (\( I_{P, Ca} \)), and intracellular Ca²⁺, similar to the AT₁ receptor modulation of \( I_K \) and \( I_{Ca} \) by Ang II (Zhu et al. 1997). These effects are consistent with the increases in neuronal activity observed following AT₁ receptor activation (Ambuhl et al. 1992; Yang et al. 1992). Combined with our present findings, we suggest that by potentiating \( I_{Ca} \) and diminishing \( I_K \) and \( I_A \), Ang II increases the excitability of neurons from the hypothalamus and brain stem. This too is consistent with our recent observation that Ang II increases the spontaneous firing rate of the cultured neurons (see companion paper).

Ang II produced three important biophysical, physiologi-
FIG. 8. Effects of Ang II on single \( I_A \) recorded from cell-attached patches. Experiments were performed under same conditions as Fig. 4. Currents were activated by driving force of +100 mV (\( V_{m0} = \) membrane potential = +100 mV). Left: control single-channel currents and ensemble average current. Middle: effects of bath application of Ang II (100 nM) on single-channel activity and ensemble average current. Right: recovery on washout of Ang II. This is representative of 4 experiments.

FIG. 9. Ang II decreases open probability (\( NP_o \)) of A-type K\(^+\) channels. Single-channel analysis was performed on recordings from Fig. 8. \( NP_o \), in control, in presence of Ang II (100 nM), and after washout was 0.21, 0.10, and 0.16, respectively. Note that Ang II did not alter single-channel amplitude of A-type K\(^+\) channels.
The similarity in steady-state inactivation and differences in inactivation kinetics during a voltage step in different neuronal preparations may be due to specific molecular mechanisms involving the coexistence of N-type and C-type inactivation mechanisms in A-type $K^+$ channels (Armstrong and Bezanilla 1977; Baukrowtz and Yellen 1995; Choi et al. 1991; Grissom and Cahalan 1989; Yellen et al. 1994; Zagotta et al. 1990).

The conductance of single A-type $K^+$ channels recorded under the cell-attached configuration is related to the transmembrane potassium gradient. It is well accepted that the A-type $K^+$ channels have a small conductance ($\sim 15-20$ pS, Fig. 4) (Rudy 1988) in a physiological $K^+$ gradient ([K$^+$]$_{in}$ = 5.4 mM). Finally, pharmacologically $I_A$ and single A-type $K^+$ channels from neurons of the hypothalamus and brain stem resemble other neurons in their 4-AP sensitivity. Kasai et al. (1986) reported that 4-AP blocked A-type $K^+$ channels from the inside of the cell by diffusing through the membrane. We also observed that bath application of 4-AP could inhibit $I_A$ (Fig. 1) and single A-type $K^+$ channel activity recorded in outside-out patches (Fig. 5).

In summary, we have characterized a TEA-resistant, 4-AP-sensitive $I_A$ and the single channel underlying it in rat cultured neurons with the use of whole cell, outside-out patch, and cell-attached patch configurations. We have also shown that Ang II, through activation of AT$_1$ receptors, inhibits $I_A$ and single A-type $K^+$ channels. The Ang II regulation of neuronal $I_A$ is one of the bases for its physiological actions through changes in action potential firing patterns in the brain. Future experiments will include the elucidation of the sign of the transduction mechanism(s) involved in Ang II regulation of $I_A$.

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