Sequential and opposite regulation of two outward K⁺ currents by ET-1 in cultured striatal astrocytes

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Bychkov, R., J. Glowinski, and C. Giaume. Sequential and opposite regulation of two outward K⁺ currents by ET-1 in cultured striatal astrocytes. Am J Physiol Cell Physiol 281: C1373–C1384, 2001.—In the brain, astrocytes represent a major target for endothelins (ETs), a family of peptides that can be released by several cell types and that have potent and multiple effects on astrocytic functions. Four types of K⁺ currents (I_K) were detected in various proportions by patch-clamp recordings of cultured striatal astrocytes, including the A-type I_K, the inwardly rectifying I_K1R, the Ca²⁺-dependent I_K (I_KCa), and the delayed-rectified I_K (I_KDR). Variations in the shape of current-voltage relationships were related mainly to differences in the proportion of these currents. ET-1 was found to regulate with opposite effects the two more frequently recorded outward K⁺ currents in striatal astrocytes. Indeed, this peptide induced an initial activation of I_KCa (composed of SK and BK channels) and a delayed long-lasting inhibition of I_KDR. In current-clamp recordings, the activation of I_KCa correlated with a transient hyperpolarization, whereas the inhibition of I_KDR correlated with a sustained depolarization. These ET-1-induced sequential changes in membrane potential in astrocytes may be important for the regulation of voltage gradients in astrocytic networks and the maintenance of K⁺ homeostasis in the brain microenvironment.

Endothelins (ETs) constitute a family of peptides that includes at least the following three isoforms: ET-1, ET-2, and ET-3. Originally described in porcine endothelial cells as a very potent vasoconstrictor, ET-1 is the most thoroughly studied member of this family (58–60). ETs exert multiple effects on vascular and nonvascular tissues and have been implicated in several physiological functions in the cardiovascular, endocrine, pulmonary, renal, and nervous system. ET precursors and receptors are present in situ in the mammalian brain, including the human brain (53). ET responses are mediated via at least two ET receptor subtypes, ET₁A and ET₁B, which are coupled to heterotrimeric G proteins and widely distributed in the brain (see Ref. 52). ET-1 and ET-3 isoforms are present in vivo in brain endothelial cells (59), some neurons (19), and reactive astrocytes (27). Primary cultures of astrocytes provide a suitable model for investigating the ET system, since these cells naturally contain ET-1 and ET-3 (18, 20), express G protein-coupled ET receptors (31, 32), and possess ET-converting enzyme activity (15).

Although less documented than in peripheral tissues, the biological effects of ETs in the central nervous system have also been investigated, mainly in astrocytes. In these glial cells, effects of ETs are widespread, including mobilization of various transduction pathways, enhancement of glucose uptake (52) and gluta-
mate efflux (44), increase of c-fos and nerve growth factor expression, regulation of ionic channel activity (50), stimulation of mitogenesis (51) and proliferation (48), inhibition of gap junction-mediated intercellular communication (4, 20), and triggering of intercellular Ca²⁺ waves (54). The significance of these responses on astrocytic function is still unclear (17), but ETs are known to be released by reactive astrocytes and may be involved in various disorders (35, 37, 42). Indeed, ET immunoreactivity and ET₁ receptor expression are increased significantly in astrocytes after brain injury (41, 43). In addition, ET levels are also enhanced in several neurological disorders, including Alzheimer's disease (60), brain inflammation (36), virus infection (27), subarachnoidal hemorrhage (32), and ischemia (2, 57). Interestingly, ET receptor antagonists exert therapeutic effects in animal models of cerebrovascular diseases (see for reviews Refs. 2 and 37). Undoubtedly, an increased understanding of the basic effects of ETs on astrocytic properties should clarify how these peptides intervene in these pathological and experimental situations.

Astrocytes from either primary culture or acute brain slices express a large spectrum of ionic channels, depending on their brain region of origin and their reactive state. Although much information is already available on the regulatory role of ETs on ionic channels in cardiovascular, endocrine, and muscular tissue, little is known concerning their influence on the central nervous system. Studies characterizing membrane potential revealed for the first time that astrocytes can be...
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a target for ETs (24). However, the ionic channel subtypes contributing to ET-glial responses have been poorly characterized so far. Accordingly, this study was undertaken to determine which K⁺ channels are regulated by ET-1 in cultured astrocytes from the rat striatum.

METHODS

Cell culture. Primary cultures were prepared as described previously (54). Briefly, pregnant OFA rats (IFAA Credo, Lyon, France) were killed by prolonged exposure to high concentrations of carbon dioxide. Embryos (18 days old) were removed rapidly from the uterus and placed in PBS supplemented with glucose (33 mM). Striata were dissected and mechanically dissociated in PBS-glucose solution. Cells were plated on 12-mm glass coverslips (3 x 10⁵ cells/coverslip) coated with poly-L-ornithine (15 μg/ml). The culture medium consisted of a 1:1 mixture of MEM and F-12 nutrient (GIBCO, Gaithersburg, MD) supplemented with glutamine (2 mM), NaHCO₃ (13 mM), HEPES (5 mM, pH 7.4), glucose (33 mM), penicillin-streptomycin (5 IU/ml and 5 mg/ml, respectively), and 10% Nusereum (Collaborative Research, Bedford, MA). Once plated, cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The culture medium was changed one time per week. On day 8, cytosine arabinoside (2 μM) was added for 60 h to prevent proliferation of fibroblasts and microglia. Cells were studied between 10 and 20 days of culture. At this time, >95% of the cells stained positive for glial fibrillary acidic protein, as revealed by indirect immunofluorescence (INC Biochemicals, Costa Mesa, CA).

Electrophysiology. Whole cell K⁺ currents were measured using whole cell, outside-out, or perforated patch-clamp configurations. The external solution contained (in mM) 140 NaCl, 1.8 CaCl₂, 1 MgCl₂, 5.4 KCl, and 10 Na-HEPES (pH 7.4), whereas recording pipettes (resistance 3–8 MΩ) were filled with a solution containing (in mM) 140 NaCl, 1.8 CaCl₂, 1 MgCl₂, 5.4 KCl, and 10 Na-HEPES (pH 7.4), whereas recording pipettes (resistance 3–8 MΩ) were filled with a solution containing (in mM) 80 potassium aspartate, 40 KCl, 20 NaCl, 1 MgCl₂, 3 MgATP, 10 EGTA, and 5 K-HEPES (pH 7.4). EGTA was balanced with Ca²⁺ to provide 100, 200, or 500 nM free Ca²⁺. The cesium pipette solution used to block K⁺ channels contained (in mM) 140 NaCl, 1.8 CaCl₂, 1 MgCl₂, 5.4 KCl, and 10 Na-HEPES (pH 7.4), whereas recording pipettes (resistance 3–8 MΩ) were filled with a solution containing (in mM) 80 potassium aspartate, 40 KCl, 20 NaCl, 1 MgCl₂, 3 MgATP, 10 EGTA, and 5 K-HEPES (pH 7.4). EGTA was balanced with Ca²⁺ to provide 100, 200, or 500 nM free Ca²⁺. The cesium pipette solution used to block K⁺ channels contained (in mM) 80 potassium aspartate, 40 KCl, 20 NaCl, 1 MgCl₂, 3 MgATP, 10 EGTA, and 5 K-HEPES (pH 7.4). When Ca²⁺-free solution was used, Na⁺ substituted for the Ca²⁺. Solutions were superfused in the recording chamber (1 ml) by gravity flow with a renewal time of ~45 s. Drugs were applied using thin tubes connected to a 2-ml syringe, and superfusion was stopped. In the perforated patch-clamp experiments, whole cell access was achieved by nystatin within 5–10 min after seal formation. Nystatin (Sigma Chemicals, St. Louis, MO) was dissolved in DMSO and was added to the pipette solution (final concentration, 50–100 μM).

Voltages and currents were recorded at 5–10 kHz using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Signals were filtered at 1 kHz, digitized using a Digidata 1200 interface (Axon Instruments, Foster City, CA), and analyzed using the pCLAMP software (version 6). Series resistance and cell capacitance were calculated from the uncompensated capacitive transients using hyperpolarizing step pulses (10 mV, 10 ms) and by adjusting the amplifier’s series resistance and whole cell capacitance controls to eliminate current transients. Cell membrane input resistance was measured using small hyperpolarizing voltage pulses (10 mV, 10 ms) from a holding potential of ~40 mV. All experiments were performed at room temperature (20–24°C).

Data analysis. All values are given as means ± SE; n represents the number of cells tested. Statistical analysis was performed using a one-way ANOVA test, and a value of P < 0.05 was considered statistically significant.

RESULTS

In agreement with previous publications, we observed that rat striatal astrocytes in primary cultures exhibit a great heterogeneity of K⁺ current expression (1, 3, 13, 33, 38, 45, 46). Indeed, among 75 astrocytes initially tested by voltage steps or ramps, 16% were characterized by a linear current-voltage (I-V) relationship, whereas the remaining astrocytes exhibited various patterns of I-V curves, indicating voltage-dependent properties. Generally, four types of K⁺ currents were recorded using the perforated-patch and the whole cell configuration, including slow-inactivating delayed-rectifier-type K⁺ currents (Iₖ,DR), inwardly rectifying K⁺ currents (Iₖ,IR), Ca²⁺-dependent K⁺ currents (Iₖ,CA), and fast-inactivating A-type K⁺ currents (Iₖ,A). Variations in the shape of the I-V relationships were related mainly to differences in the proportions of these currents.

Voltage-dependent outward K⁺ currents in cultured striatal astrocytes. To determine whether K⁺ was the major charge carrier of the net outward current, K⁺ was replaced by Cs⁺ (120 mM CsCl) and tetraethylammonium (TEA; 10 mM) in the internal pipette solution. Under these conditions, outward currents were markedly decreased (15 ± 8% of the control value, n = 7), suggesting that these currents were carried mainly by K⁺. The contribution of Cl⁻ to these outward currents was also tested using Cl⁻ channel blockers. In the presence of either 5-nitro-2,3-phenylpropylamino benzoic acid (100 μM) or DIDS (100 μM), the outward currents recorded at +100 mV were reduced only slightly (91 ± 2% and 94 ± 1% of the control value, respectively, n = 5), suggesting only a minor contribution of the Cl⁻ currents under our recording conditions. Further support for K⁺ as the major charge carrier of the outward currents was obtained from examining tail currents, which were recorded at various repolarizing potentials after a prepulse step to +80 mV. The tail currents indicated an average reversal potential of −78 ± 4 mV (n = 7), which is close to the calculated Nernst equation for the K⁺ reversal potential (E_K = −83 mV).

As shown in Figs. 1 and 2, the outward K⁺-currents were recorded in response to step pulses (±150 mV, 10-mV increments, 300–500 ms) and voltage-ramp (±150 mV, 800 ms) applications, which were both recorded from a holding potential of −80 mV. In ~30% of the recordings (19 of 75 cells), depolarizing pulses generated Iₖ,A-like currents that inactivated rapidly (data not shown). In addition, all astrocytes characterized by voltage-dependent properties exhibited slowly inactivating currents. On the basis of voltage dependence and pharmacological testing of K⁺ currents, two main types of astrocytes were distinguished. The first type (29 of 124 cells) exhibited an I-V relationship fitted by a single exponential function and was charac-
terized by a threshold of activation at $-16 \pm 3$ mV ($n = 12$; Fig. 1A). The outward currents elicited by positive step pulses increased continuously without a saturation plateau. Inward currents resulting from the activation of $I_{K_{IR}}$ at negative steps were inhibited by 100 μM Ba$^{2+}$ (Fig. 1A and B). Subsequent applications of apamin (100 nM) slightly decreased the amplitude of the outward K$^+$ currents over the entire range of potentials investigated (Fig. 1, A and B). Furthermore, cumulative applications of iberiotoxin (100 nM) drastically decreased the amplitude of the outward K$^+$ currents, whereas iberiotoxin and apamin together induced saturation of the resistant K$^+$ outward currents. The second type of astrocyte (68 of 124 cells) was characterized by a more complex current profile composed of at least two components. The threshold of activation for this type of astrocyte was detected at $-42 \pm 5$ mV ($n = 17$). In addition, at potentials more positive than +50 mV, the current amplitude increased continuously after exhibiting a plateau in the I-V relationship (Fig. 2A). In these cells, $I_{K_{IR}}$ were inhibited by 100 μM Ba$^{2+}$, whereas a subsequent application of iberiotoxin (100 nM) decreased the amplitude of the outward K$^+$ currents at potentials more positive than +20 mV (Fig. 2, A and B). Furthermore, cumulative applications of apamin (100 nM) linearly decreased the amplitude of the K$^+$ outward currents at all ranges of potential investigated. The K$^+$ currents resistant to iberiotoxin and apamin exhibited a saturation plateau face with a slightly pronounced inactivation of the K$^+$ current at positive potentials. Finally, 27 cells showed intermediary and more complex I-V relationships.
addition to step pulses, voltage ramps (800 ms, ±150 mV) were used to establish the I-V relationship of striatal astrocytes. Comparison of averaged I-V curves obtained using the two protocols indicated that they differed slightly in their amplitude, whereas their shapes were similar (Figs. 1 and 2).

Differences in the two main I-V relationships were analyzed further (20 cells in each case) by computing conductance (dI/dV) as a function of membrane potential (Figs. 1C and 2C, insets). The first category of astrocyte (Fig. 1), characterized by a continuous increase in current amplitude elicited by step pulses or voltage ramps, exhibited a single maximum of conductance (i.e., the derivative of the current to voltage) at +94 ± 6 mV (Fig. 1C, inset). The second category of astrocyte (Fig. 2), characterized by a plateau phase around +40 mV in the I-V relationship and by a continuous increase in the current amplitude at more positive potentials, exhibited two peaks of conductance at +47 ± 4 and +100 ± 8 mV (Fig. 2C, inset). From the plots, astrocytes with an I-V relationship that resulted in a conductance curve with one maxima were named S-shaped conductance astrocytes (S-G-V), whereas astrocytes with an I-V relationship that resulted in a...
conduction curve with two maxima were called N-shaped conduction astrocytes (N-G-V).

The two classes of astrocytes had different sensitivities to the K⁺ channel blockers TEA and 4-aminopyridine (4-AP). Indeed, in S-G-V astrocytes, TEA (0.1–7 mM) decreased in a concentration-dependent manner the amplitude of outward K⁺ currents elicited by ramp voltages mainly at potentials more positive than +50 mV (Fig. 3A). The TEA-induced inhibition of the total outward current (measured at +100 mV) was further characterized by plotting the percentage of inhibition (compared with the control value) against TEA concentration (Fig. 3B). The progressive decline of outward K⁺ currents in the presence of increasing TEA concentrations was well fitted by a sigmoid function consistent with a 1:1 binding of TEA to its binding site, with an IC₅₀ of 475 mM and a slope of 1.59. In contrast, 4-AP used in a concentration ranging from 0.1 to 5 mM had little or no inhibitory effect on S-G-V astrocytes (n = 9, data not shown), whereas N-G-V astrocytes were sensitive to 4-AP at negative and positive potentials. As shown in Fig. 3C, 4-AP reduced in a concentration-dependent manner outward currents elicited by voltage ramps in these astrocytes. Concentration-response curves for the inhibition of outward K⁺ currents measured at +20 mV were constructed. The averaged data were well fitted by a sigmoid function, consistent with a 1:1 binding of 4-AP to its binding site and an IC₅₀ of 1.5 mM. Even at higher concentrations (5–10 mM), 4-AP reduced only 20% of the total outward current (measured at +100 mV). However, the coapplication of 4-AP (5 mM) and TEA (5 mM) resulted in an 83 ± 9% block of the outward K⁺ currents in N-G-V astrocytes (n = 12), indicating the presence of at least two types of K⁺ currents in these cells.

Action of ET-1 on outward K⁺ currents. The effects of ET-1 on the major outward K⁺ currents were investigated in S-G-V and N-G-V astrocytes. ET-1 was used at 0.1 μM, a concentration known to induce maximal responses on the signal transduction pathway in cultured astrocytes (4, 20, 24, 31, 32, 50, 52).

In 14 of 19 S-G-V astrocytes, ET-1 transiently increased the nonlinear K⁺ outward currents (Fig. 4A). This ET-1-induced increase in the amplitude of K⁺ outward currents reached 189 ± 38% (n = 14) at +50 mV. The ET-1-activated current remained nonlinear and returned to 92 ± 4% of the control value 19 ± 7 s (n = 14) after the beginning of the application (Fig. 4A). In this class of astrocytes, ET-1 elicited a weak voltage-independent outward net current insensitive to iberiotoxin (Fig. 4A). Finally, pretreatment of S-G-V astrocytes with iberiotoxin (0.1 μM) prevented the stimulatory effect of ET-1 on the voltage-dependent K⁺ outward current (n = 5; data not shown).

In 23 of 45 N-G-V astrocytes, shortly (first 1 min) after the beginning of its application, ET-1 progressively inhibited by 39 ± 7% (n = 8) the outward K⁺ current compared with the control value. After this biphasic effect of ET-1, I-V curves of the cells were transformed from an N-G-V to an S-G-V shape computed slope conductance (Fig. 4B). In 8 of these 45 cells, the transitory stimulatory effect of ET-1 did not occur, with only the reduction of the voltage-gated K⁺...
 current being observed. Furthermore, pretreatment of N-G-V astrocytes with apamin (0.1 μM) prevented the initial transient linear current evoked by ET-1 \( (n = 6) \). When toxin was applied after the ET-1-induced transient changes of \( K^+ \) currents, a further decrease \( (38 \pm 8\% \text{ of the control value}) \) in the amplitude of the outward \( K^+ \) currents was observed. Finally, a larger inhibitory response \( (19 \pm 6\% \text{ of the control value}, n = 8) \) occurred under the coapplication of apamin (0.1 μM) and iberiotoxin (0.1 μM) after ET-1 treatment (Fig. 4B).

Because ET-1 triggers complex intracellular \( Ca^{2+} \) concentration \( ([Ca^{2+}]_i) \) elevations in striatal astrocytes \( (4, 55, 56) \), whole cell recordings were also performed in N-G-V cells with 10 mM EGTA in the recording pipette solution to prevent \( [Ca^{2+}]_i \) transients. Outward \( K^+ \) currents were generated by either voltage ramps \( (+100 \text{ mV}) \) or voltage steps \( (10 \text{ mV}) \) applied from \(-80 \text{ to } +100 \text{ mV} \), starting from a holding potential of \(-80 \text{ mV} \) (Fig. 5, A and B). Under these conditions, ET-1 application \( (0.1 \mu M) \) inhibited an outward current having typical kinetics of \( I_{K_{DR}} \) (Fig. 5, A, inset, and B). This ET-1-induced inhibition of \( I_{K_{DR}} \) was concentration dependent (Fig. 5C) and was observed at peak and steady-state levels. \( IC_{50} \) and curve slope, estimated from the best fit of the logistic function, were 112 nM and 1.72, respectively.

The voltage dependence of steady-state inactivation of \( I_{K_{DR}} \) was studied using a double-pulse protocol. The extent of channel inactivation was assessed by measuring the peak outward \( K^+ \) current \( (+50 \text{ mV}, 500 \text{ ms}) \) after holding the membrane potential for 20 s at values ranging from \(-80 \text{ to } +20 \text{ mV} \) with 10-mV increments (Fig. 5, D and E). Typically, as indicated by plotting \( I/I_{\text{max}} \) vs. the membrane potential, the initial depolarization increased the inactivation of the outward \( K^+ \) current (Fig. 5D). The inactivation curve of the outward \( K^+ \) current was well fitted by the Boltzmann equation as follows: \( II_{\text{max}} = (I_{\text{max}} - I_{\text{min}})/[1 + \exp(V - V_i)/k] + I_{\text{min}} \), where \( I \) (pA) is the amplitude of the outward current elicited by the test pulse \( V \) (mV), preceded by the conditioning prepulse; \( I_{\text{max}} \) (pA) is the amplitude of the current elicited by a test pulse from a conditioning prepulse of \(-80 \text{ mV}; I_{\text{min}} \) (pA) is the non-inactivating current component; \( V_h \) (mV) is the value of the conditioning potential leading to 50% inactivation of the \( K^+ \) current; and \( k \) is the steepness factor characterizing the voltage sensitivity of the channels.

Effects of ET-1 on single-channel activities. Single-channel recordings were performed in outside-out configuration with internal and external solution containing 150 and 5.4 mM \( K^+ \), respectively, and \( Ca^{2+} \) concentration in the pipette solution was fixed at 200 nM. Under these conditions, two main types of noninactivating \( K^+ \) channel activities were distinguished by comparing currents recorded at potentials ranging from \(-40 \text{ to } +90 \text{ mV} \). On the basis of their distinct amplitudes, two channel populations were observed in either the same (Fig. 6A) or in different excised patches of membrane (Fig. 7). The amplitude of these two types of unitary currents were analyzed and plotted against membrane potential to determine their unitary conductance. Plots of large unitary currents were well fitted by linear regression (Fig. 6C), and an averaged unitary conductance of \( 189 \pm 7 \text{ pS} \) \( (n = 7) \), which corresponds to characteristic BK channels, could be determined. The conductance of \( K^+ \) channels exhibiting low-amplitude currents was estimated using a linear fit from 0 to \(+40 \text{ mV} \) (Fig. 6D). The unitary \( I-V \) relationship constructed from averaged data gave an average unitary conductance of \( 21 \pm 3 \text{ pS} \) \( (n = 6) \), corresponding to the small conductance of SK channels (Fig. 6D).

Because ET-1 \( (0.1 \mu M) \) did not affect the behavior of the single channels recorded in the outside-out config-

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**Fig. 4.** Effect of endothelin (ET-1) on voltage-dependent outward \( K^+ \) currents recorded from S-G-V and N-G-V astrocytes. A: perforated-patch recording of outward \( K^+ \) currents elicited in a type S-G-V astrocyte by ramp depolarization before (0 s) and after \( (0.1 \mu M) \) ET-1 applied at the indicated times. ET-1 application induced a large and transient increase in the amplitude of the outward \( K^+ \) currents and a shift to the left of the threshold of activation. When iberiotoxin \( (100 \mu M) \) was added to the bath solution after a transitory activation of the remaining outward \( K^+ \) current was completed, the toxin drastically reduced the remaining outward \( K^+ \) currents. B: family of current traces recorded from a type N-G-V conductance astrocyte before and after ET-1 application. Note that, 60 s after the peptide application, the outward \( K^+ \) currents did not return to the control value and that the component, which is activated at negative membrane potentials in the control situation, was lacking (broken line). Apamin \( (0.1 \mu M) \) added to the bath solution elicited further inhibition of the outward \( K^+ \) currents, whereas iberiotoxin \( (0.1 \mu M) \) added with apamin \( (0.1 \mu M) \) blocked the remaining current.

**Fig. 5.** Voltage dependence of steady-state inactivation of \( I_{K_{DR}} \) (A). Inactivation curve of the outward \( K^+ \) current was well fitted by the Boltzmann equation as follows: \( II_{\text{max}} = (I_{\text{max}} - I_{\text{min}})/[1 + \exp(V - V_i)/k] + I_{\text{min}} \), where \( I \) (pA) is the amplitude of the outward current elicited by the test pulse \( V \) (mV), preceded by the conditioning prepulse; \( I_{\text{max}} \) (pA) is the amplitude of the current elicited by a test pulse from a conditioning prepulse of \(-80 \text{ mV}; I_{\text{min}} \) (pA) is the non-inactivating current component; \( V_h \) (mV) is the value of the conditioning potential leading to 50% inactivation of the \( K^+ \) current; and \( k \) is the steepness factor characterizing the voltage sensitivity of the channels.
from 34 N-G-V astrocytes. The current traces in Fig. 6 imposed on the whole cell current could be recorded in 13 these recording conditions, single-channel activity super-

The current inhibited by the peptide ($I_{\text{con}} - I_{\text{ET-1}}$) is shown in current trace on right. C: dose-response curve showing the inhibitory effect of ET-1 on outward K$^+$ currents in response to +50-mV voltage steps. Current plot corresponds to the normalized current expressed as the percentage of the control value. Aver-

aged data (means ± SE from 4–8 cells) were fitted with logistic functions with an EC$_{50}$ value and slope of 1.11 μM and 1.7, respectively. D: inactivation of outward K$^+$ currents in the presence and absence of ET-1. Plots of the relationship between peak outward K$^+$ currents recorded at a test potential of +50 mV and membrane potential (preconditioning potential) before (●) and after (▲) ET-1 application ($n = 5$). The membrane po-

tential was held at −80 mV, stepped to a preconditioning voltage (90 s) ranging from −80 to 0 mV (10-mV increments), and then depolarized to +40 mV (500 ms). Data obtained before and after application of ET-1 were fitted by curves describing the Boltzmann function, with midpoints of inactivation ($V_{1/2}$) of −30.6 ± 1.6 and −21.5 ± 2.5 mV, and steepness factor ($k$) was 11.2 ± 0.2 and 9.7 ± 0.6 mV, respectively. E: typical K$^+$ currents elicited by test pulse to +50 mV under control conditions and after appli-

cation of ET-1. All recordings were obtained in the whole cell configuration.

For further analyses, the background resting current was subtracted and digitally filtered at 400 Hz while ET-1 was applied in Ca$^{2+}$-free solution. ET-1 (0.1 μM) application increased the open-state probability of SK and BK channels from 0.24 ± 0.03 to 0.81 ± 0.08 (n = 7) and 0.15 ± 0.07 to 0.64 ± 0.017 (n = 5), respectively. In addition, maximal time opening of single SK and BK channels was significantly enhanced from 81 ± 12 to 394 ± 41 ms (n = 7) and 31 ± 4 to 58 ± 9 ms (n = 5), respectively (Fig. 7, A–D).

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but not iberiotoxin (0.1 μM, data not shown) inhibited the ET-1-induced activity of SK channels. In contrast, BK channels activated by ET-1 were highly sensitive to iberiotoxin (0.1 μM, Fig. 7D). BK channels were also found to be activated differently than SK channels in response to the rise in [Ca$^{2+}$]$_i$. Indeed, because of the simultaneous opening of several BK channels (from 4 to 8 channels), which resulted in current transients, BK channels exhibited a highly cooperative behavior under ET-1 application (Fig. 7D). This cooperative behavior was not observed for SK channels.

Changes in membrane potential elicited by ET-1 in astrocytes. Finally, the effect of ET-1 on membrane potential of cultured striatal astrocytes was investigated in current-clamp mode. As previously reported (16, 33, 49), cultured astrocytes exhibited a wide heterogeneity of resting membrane potentials, ranging from −78 to −29 mV, with an average value of −48 ± 18 mV (n = 35). Recordings from 21 astrocytes indicated that ET-1 (0.1 μM) evoked several patterns of changes in membrane potential. Indeed, addition of peptide triggered either a transient membrane hyperpolarization of −28 ± 6 mV (n = 6; Fig. 8A), a sustained depolarization of −28 ± 3 mV (n = 7; Fig. 8B), or a biphasic change in membrane potential characterized by a transient hyperpolarization followed by a sustained depolarization (n = 4; Fig. 8C). However, no modification in membrane potential could be observed in 4 of the 21 cells exposed to ET-1.

Additional experiments were made to determine the relationship between the ET-1-induced activation of $I_{KCa}$ and inhibition of $I_{KDR}$ and the various effects of this peptide on membrane potential. Several observations suggested that transient hyperpolarization resulted from the activation of $I_{KCa}$ linked to [Ca$^{2+}$], elevation. First, ionomycin application (1 μM) in the absence of external Ca$^{2+}$ was always followed by hyperpolarization (30 ± 5 mV, n = 5). Second, although no
change in membrane potential was observed when iberiotoxin (0.1 μM) was added to the bath solution \( (n = 8) \), ET-1 application showed no effect \( (n = 3) \) or induced only a 22 ± 2-mV depolarization \( (n = 3) \). Third, when cells were treated with apamin (0.1 μM), which alone induced a slight depolarization of 11 ± 3 mV \( (n = 7) \), ET-1 (0.1 μM) evoked an additional depolarization of 18 ± 4 mV \( (n = 7) \) of 21 cells was the result of inhibition of this current by ET-1. Indeed, this \( I_{KD} \) blocker, which alone depolarized the cells by 31 ± 2 mV \( (n = 5) \), prevented the ET-1 (0.1 μM)-induced depolarization \( (n = 4) \).

**DISCUSSION**

The present study demonstrates that ET-1 exerts sequential and opposite effects on the outward \( K^+ \) currents of cultured astrocytes from the rat striatum. Indeed, this peptide first activates two subtypes of \( Ca^{2+} \)-dependent channels contributing to \( I_{Ca} \), \( BK \) and \( SK \) channels, according to their pharmacological and biophysical properties. Second, ET-1 reduces total outward \( K^+ \) currents through a prolonged inhibition of \( I_{KD} \) channels. These effects were observed on the same cells or separately on distinct cells. Astrocyte heterogeneity is likely responsible for this variety of responses. Because \( I_{Ca} \) and \( I_{KD} \) are involved in several cell functions, the regulatory effects of ET-1 could account for some of the central effects of ETs.

As shown, astrocytes express at least four types of \( K^+ \) currents \( (8, 30, 46) \). Three currents, which are voltage-dependent and \( Ca^{2+} \)-independent, were identified as \( I_{KA} \), \( I_{KD} \), and \( I_{KR} \) by their pharmacological and kinetic properties \( (1, 3, 7, 34, 40, 46) \). These three \( K^+ \) currents have been recorded extensively in either...
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Fig. 8. Three different effects (A–C) of ET-1 on membrane potential. All experiments were performed in the perforated-patch configuration. Broken line indicates the resting membrane potential before ET-1 application (0.1 μM).

primary cultures or acute brain slices (9, 13, 14, 38, 39). Finally, an $I_{K_{Ca}}$ has also been described in astrocytes (30, 50). This latter current results from the contribution of BK channels, characterized by a large unitary conductance (>80 pS), sensitivity to voltage, and blockade by charrybotoxin and iberiotoxin (11, 12, 25), and from SK channels, characterized by a smaller unitary conductance (<30 pS), a weak sensitivity to voltage, and blockade by apamin (6, 16, 23).

These four K⁺ currents have already been described in several studies on astrocytes. Depending on the preparation (primary cultures or brain slices) and the brain region investigated (1, 3, 40, 46), the contribution of these four K⁺ currents was estimated previously and indicated the existence of astrocyte heterogeneity. In cultured striatal astrocytes, variation in the shape of the $I-V$ relationship was related to differences in the proportions of the channel subtype. In fact, the following two distinct forms of $I-V$ relationships were distinguished: 1) S-G-V astrocytes, characterized by high sensitivity to TEA and iberiotoxin, and 2) N-G-V astrocytes, in which $I_{K_{DR}}$ was the prevalent current under resting conditions. Moreover, no morphological differences between the astrocytes were correlated with the variations in the $I-V$ relationship.

Generally, SK channels are distinguished from BK channels by their higher sensitivity to intracellular Ca²⁺ and their slower and lower responses to changes in potential (6, 34). In striatal astrocytes, transient increases in [Ca²⁺]ᵢ induced by ET-1 enhanced the activity of BK and SK channels with different profiles. The open time of SK channels was increased much more than that of BK channels. Several BK channels, but not SK channels, were opened simultaneously within a short duration (cooperative opening). Because Ca²⁺ signaling plays a pivotal role in astrocyte function (21, 54, 56), the occurrence of SK and BK channels in these cells may be engaged in physiological processes. Indeed, in astrocytes, SK channels at negative potentials (from -40 to 0 mV) and at moderate [Ca²⁺]ᵢ, increases might maintain long-lasting potential-independent hyperpolarizations. Also, SK channels are generally involved in the same process, which follows repetitive action potential firing in neurons (6, 26), a phenomenon that occurs in astrocytes in pathological conditions (8, 47). BK channels with fast voltage-dependent kinetics operate at potentials more positive than 0 mV and provide a negative feedback to rapidly hyperpolarize the cell. Moreover, the cooperative opening of BK channels usually results in the appearance of spontaneous transient outward currents (STOCs; see Refs. 5 and 11), but these are not observed in striatal astrocytes. As shown for cardiomyocytes and skeletal and smooth muscle cells, besides the expression of BK channels, STOCs generally require the close association of other defined cellular elements, including voltage-gated Ca²⁺ channels, sarcoplasmic reticulum ryanodine receptor channels, and Na⁺/Ca²⁺ exchangers (5, 10). The absence of voltage-gated Ca²⁺ channels and the weak ryanodine response in striatal astrocytes (21) may contribute to the lack of STOCs in these cells. Alternatively, the activation of these two types of Ca²⁺-dependent K⁺ channels could occur during the propagation of intercellular Ca²⁺ waves (22). Thus SK and BK channels could constitute a target for these waves. These channels could be activated not only in cells directly stimulated by ET-1 but also in neighboring astrocytes to which the Ca²⁺ waves propagate.

Our results indicate that ET-1 transiently activates $I_{K_{Ca}}$ in cultured striatal astrocytes. A similar observation was made after ionomycin application in the absence of external Ca²⁺ (data not shown), indicating that Ca²⁺ released from internal stores activates SK and BK channels in these cells. Interestingly, in response to ET-1 (0.1 μM) application, the activation of $I_{K_{Ca}}$ (the present study) and the inositol trisphosphate-evoked increase in [Ca²⁺]ᵢ (4, 21, 54) have the same duration (30 s). From these observations, it is expected that other peptides, neurotransmitters, or hormones that induced similar increase in [Ca²⁺]ᵢ (see Ref. 22) may also activate SK and BK channels in astrocytes. ET-1 also reduced $I_{K_{DR}}$, but this effect was long lasting and occurred with a delay. The differing time courses for the ET-1 effects on K⁺ currents occurred not only when both responses were observed on the same cell but also when the responses were recorded separately on distinct cells. This suggests that these two ET-1-mediated regulatory processes are independent. Consequences on resting membrane potential of the sequential regulations of Ca²⁺-dependent K⁺ channels and $I_{K_{DR}}$ by ET-1 were determined in
experiments carried out using current-clamp mode. Because membrane potentials were monitored in the perforated-patch configuration with imposed ion gradients, the measured values may differ from the physiological situation measured by intracellular microelectrodes (24). However, the relative time course changes in membrane potential induced by ET-1 and their sensibility to the various blockers used allowed us to dissect the participation of identified K⁺ channels. The following three modalities of potential changes were observed: a biphasic effect resulting from a transient hyperpolarization followed by a sustained depolarization, a single transient hyperpolarization, or a prolonged depolarization. The similarity in the time courses of the ET-1 responses suggested that the hyperpolarization results from the activation of \( I_{K_{Ca}^+} \), while the subsequent depolarization corresponds to the inhibition of \( I_{K_{DR}} \). Further supporting this statement, the ET-1-induced hyperpolarization and depolarization were prevented by the K⁺ and Ca²⁺-dependent channel blockers and 4-AP \( (I_{K_{DR}} \) blocker), respectively.

All of these observations were obtained from primary cultures. In the future, they should be complemented by using a more integrated preparation, such as acute brain slices. However, these results can be discussed in the light of previous studies indicating that ETs can affect several astrocytic functions. Indeed, ETs exert several effects in astrocytes, including an increase in glucose uptake (52), mitogenic and proliferative responses (28, 29, 41, 48), and an initiation followed by an inhibition of the propagation of intercellular Ca²⁺ waves (4, 54). Some of these responses could be attributed to the ET-1-induced regulation of K⁺ outward currents, since several of these currents were found to contribute to astrocytic functions. For instance, \( I_{K_{DR}} \) seems to be critical for astrocytic proliferation (17). However, astrocytic proliferation induced by ETs should not be related directly to \( I_{K_{DR}} \) regulation. In fact, this current is upregulated in reactive gliosis, whereas proliferation is inhibited and scar repair is delayed by the \( I_{K_{DR}} \) blocker 4-AP. On the other hand, the ET-1-induced inhibition of \( I_{K_{DR}} \) could affect K⁺ homeostasis in the following two ways: either by modulating K⁺ buffering or by affecting the other voltage-sensitive channels through changes in membrane potential. Indeed, both \( I_{K_{DR}} \) and \( I_{K_{DR}} \) are thought to be involved in K⁺ redistribution and elimination, a process referred to as “spatial buffering” (see Ref. 46). Alternatively, ET-1 could modify the shape of “glial spikes,” since in astrocytes, according to Bordey and Sontheimer (8) and Sontheimer and Ritchie (47), \( I_{K_{DR}} \) contributes to the slow afterhyperpolarization that follows the depolarization evoked by current injections. Finally, because ET-1 is presumably produced preponderantly by endothelial cells, it is likely that perivascular astrocytes contributing to the brain-blood barrier constitute a preferential target of this peptide. The sequential regulation of K⁺ channels reported here could also be involved in the regulation of K⁺ exchange between the circulating blood and the extracellular space in the central nervous system, which could affect the activity of local neuronal networks.

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