Increased Expression of \( \text{Ca}^{2+} \)-Sensitive \( K^+ \) Channels in the Cerebral Microcirculation of Genetically Hypertensive Rats
Evidence for Their Protection Against Cerebral Vasospasm

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Abstract—The \( \text{Ca}^{2+} \)-sensitive \( K^+ \) channel (\( K_{\text{Ca}} \) channel) plays a key role in buffering pressure-induced constriction of small cerebral arteries. An amplified current through this channel has been reported in vascular smooth muscle cells obtained from hypertensive animals, implying that the expression or properties of \( K_{\text{Ca}} \) channels may be regulated by in vivo blood pressure levels. In this study, we investigated this hypothesis and its functional relevance by comparing the properties, expression levels, and physiological role of \( K_{\text{Ca}} \) channels in cerebral resistance arteries from normotensive and genetically hypertensive rats. Whole-cell patch-clamp experiments revealed a 4.7-fold higher density of iberiotoxin-sensitive \( K_{\text{Ca}} \) channel current at physiological membrane potentials in spontaneously hypertensive rat (SHR) compared with Wistar-Kyoto (WKY) rat cerebrovascular smooth muscle cells (\( n = 18 \) and 21, respectively). However, additional single-channel analysis in detached patches showed similar levels of unitary conductance, voltage, and \( \text{Ca}^{2+} \) sensitivity in \( K_{\text{Ca}} \) channels from WKY and from SHR membranes. In contrast, Western analysis using an antibody directed against the \( K_{\text{Ca}} \) channel \( \alpha \)-subunit revealed a 4.1-fold increase in the corresponding 125-kD immunoreactive signal in cerebrovascular membranes from SHR compared with WKY rats. The functional impact of this enhanced \( K_{\text{Ca}} \) channel expression was assessed in SHR and WKY rat pial arterioles, which were monitored by intravital microscopy through in situ cranial windows. Progressive pharmacological block of \( K_{\text{Ca}} \) channels by iberiotoxin (0.1 to 100 nmol/L) dose-dependently constricted pial arterioles from SHR and WKY rats (\( n = 6 \) to 8). The arterioles in SHR constricted 2- to 4-fold more intensely, and vasospasm occurred in some vessels. These data provide the first direct evidence that elevated levels of in situ blood pressure induce \( K_{\text{Ca}} \) channel expression in cerebrovascular smooth muscle membranes. This homeostatic mechanism may critically regulate the resting tone of cerebral arterioles during chronic hypertension. Furthermore, the overexpression of distinct \( K^+ \) channel types during specific cardiovascular pathologies may provide for the upregulation of novel disease-specific membrane targets for vasodilator therapies. (Circ Res. 1998;82:729-737.)

Key Words: cerebral circulation ■ \( K^+ \) channel ■ vascular smooth muscle ■ hypertension ■ iberiotoxin

Cerebral blood flow is maintained at normal or near-normal levels during chronic hypertension, but the autoregulatory relationship is shifted to a higher pressure range, and vascular resistance is increased.\(^1\)-\(^3\) Although vascular remodeling of cerebral arteries resulting in reduced lumen size may protect capillary integrity and the blood/brain barrier during hypertension, it also may act to exaggerate pressure-induced constriction and amplify vasoconstrictor responses to selective stimuli.\(^1\)\(^3\) If unopposed by vascular adaptive mechanisms, local constriction or closure of small vessels involved in the pathology of hypertension may precipitate episodes of focal cortical ischemia.\(^3\) Hence, identifying local protective mechanisms in the cerebral circulation that act to maintain cerebral perfusion during hypertension may provide new insight into the pathogenic and adaptive influences that interact to regulate cerebrovascular tone during hypertensive disease.\(^3\)

In this regard, high-conductance \( K_{\text{Ca}} \) channels in cerebral smooth muscle cell membranes may have a particularly vital role in opposing pressure-induced constriction. The role of these channels as compensatory vasodilator pathways has been verified in isolated cerebral resistance arteries obtained from animals and humans.\(^4\)\(^5\) At present, little is known about the regulation of \( K_{\text{Ca}} \) channels in the cerebral circulation during cardiovascular pathologies. However, reports that the amplitude of \( K_{\text{Ca}} \) current in vascular smooth muscle cells is positively correlated with the in situ blood pressure level of the host animal suggest that this channel may be altered in hypertensive disease.\(^6\)\(^-\)\(^11\) In the present study, we specifically tested this hypothesis by comparing the current density, single-channel properties, and expression levels of \( K_{\text{Ca}} \) channels in cerebral arterial muscle membranes obtained from normotensive and hypertensive rats. Subsequently, we examined the physiological contribution of \( K_{\text{Ca}} \) channels to the...
regulation of in situ cerebral arteriolar tone in the same rat strains. Taken together, the present study provides initial evidence that an increased expression of \( \text{K}_\text{Ca} \) channels in arterial muscle membranes may provide a novel mechanism for maintaining the resting diameter of cerebral arterioles during hypertensive disorders. Shifts in vascular ion channel populations during cardiovascular diseases may provide new strategies for targeting drug treatment to specific disorders.

Materials and Methods

Experimental Animals

WKY rats and SHR. 12 to 16 weeks of age, were obtained from Taconic Farms (Germantown, NY). On the day of experiments, animals were deeply anesthetized with sodium pentobarbital (60 mg/kg IP), and their femoral arteries were cannulated with polyethylene tubing to measure mean arterial blood pressure. Average mean arterial pressure was 119±4 mm Hg in WKY rats (n=27) and 184±2 mm Hg in SHR (n=27). For Western immunoblotting, rats were decapitated after blood pressure measurement, and the brains were immediately removed and placed in cold homogenizing solution. Cerebral arteries (<100 \( \mu \text{m} \)) were rapidly dissected free, snap-frozen in liquid nitrogen, and stored at −80°C. For patch-clamp experiments, brains were placed in cold PSS, and similar cerebral arteries were carefully dissected free and then exposed to enzymatic dissociation to obtain single vascular smooth muscle cells. For instrumentation of the in situ cranial window method, final groups of SHR and WKY rats were placed in a stereotaxic station after blood pressure measurement. Both dura-open and dura-intact cranial windows permitted the performance of macroscopic \( \text{K}_\text{Ca} \) recording protocols and instrumentation previously described in detail. In some experiments, pipette tips were briefly loaded with drug-free pipette solution and then back-filled with pipette solution containing 300 nmol/L IBTX. Unitary currents were recorded immediately for 2 minutes after interface of the pipette-cell complex to obtain inside-out patches and measured again at the same membrane potential after 5 and 10 minutes to permit drug diffusion to the outside patch surface.

Evaluation of \( \text{K}_\text{Ca} \) Channel Expression by Immunoblotting

Protein samples of cerebral arteries from SHR and WKY rats were prepared as previously described for preparation of tissue homogenates. Equivalent volumes of homogenate from the cerebrovascular tissue of one WKY or SHR rat were loaded in adjacent lanes for SDS-PAGE. After size separation, proteins were transferred onto a nitrocellulose membrane and blocked with 10% nonfat dried milk in Tris-buffered saline containing 0.1% (w/v) Tween 20 (TBS-T) overnight at 4°C. Subsequently, membranes were incubated for 3 hours with a 1:1000 dilution of polyclonal rabbit anti-\( \alpha_{413-426} \), which is a sequence-directed antibody raised against amino acids 913 to 926 on the \( \alpha \)-subunit of the \( \text{K}_\text{Ca} \) channel. Membranes were then incubated for 2 hours with horseradish peroxidase-labeled goat anti-rabbit IgG in TBS-T containing 2% nonfat dried milk. A monoclonal mouse antibody raised against the structural protein \( \beta \)-actin (Sigma Chemical Co) was used as a lane-loading control. The bound antibody was detected by chemiluminescence (ECL, Amersham), and the densities of immunoreactive bands associated with anti-\( \alpha_{413-426} \) were expressed as a percentage of the \( \beta \)-actin density for each lane.

In Situ Monitoring of Pial Arteriolar Diameters in Cranial Windows

Two distinct cranial window preparations, with either opened or intact dura, were prepared for vessel observation in anesthetized ventilated SHR and WKY rats. The rat head was secured in a stereotaxic station (model 900, David Kopf Instruments), and the scalp and connective tissues were removed over the parietal cranial bone. In some experiments, the dura was carefully removed, and a cranial window frame with ports for perfusion inlet and outlet and pressure measurement was implanted as described in detail previously for the preparation of dura-open cranial windows. The window area was superfused with artificial cerebrospinal fluid (1 mL/min), and intracranial pressure was maintained between 5 and 7 mm Hg by adjusting the height of the outflow tubing. Pial vessel diameter also was monitored in dura-intact cranial windows, as described in detail recently for laser-Doppler flowmetry. Briefly, the center of the right parietal bone was thinned using a low-speed dental drill until pial vessels were visible through the remaining cranial plate. Two small burr holes were drilled on opposite sides of the window for insertion of the inflow tubing and for drainage of excess cerebrospinal fluid, respectively. The thin cranial plate in these burr holes was carefully removed under the microscope, and an incision was made in the dura, with care taken not to injure dural or pial vessels. The thinned tip of a PE-10 catheter (outer diameter, 611 \( \mu \text{m} \)) was slid carefully underneath the dura at the inlet hole and advanced to the border of the cranial window to permit superfusion of the cortical surface with artificial cerebrospinal fluid (3.8 mL/h). Both dura-open and dura-intact cranial windows permitted the infusion of IBTX onto the brain surface to examine the effect of pharmacological block of \( \text{K}_\text{Ca} \) channels on the diameter of superficial pial arterioles on the cortical surface. Images of pial arterioles were monitored with a video camera (Sanyo, VDC-2524; field size, 930×690 \( \mu \text{m} \)) after the rat was positioned under an intravital microscope, and vessel diameters were measured by an image-shearing monitor (model 608, Tektronix).
Statistics

All data are expressed as mean±SEM. Statistical comparisons between groups were made with one-way repeated-measures ANOVA with a subsequent Newman-Keuls test. Significance was accepted at a value of *P*, .05.

Results

Comparison of \( K_{Ca} \) Current Density

In Fig 1, panels A and B show macroscopic outward currents in WKY and SHR cerebral smooth muscle cells. Currents were elicited by incremental 10-mV depolarizing steps from −60 to 0 mV. IBTX (100 nmol/L) blocked a small component of outward current in the WKY cell, whereas a large current component was inhibited by IBTX in the SHR cell. C and D, Current-voltage relationships showing the effect of 100 nmol/L IBTX on peak macroscopic \( K^+ \) current in 18 WKY and 21 SHR cells, respectively. Shaded areas depict the current component sensitive to block by IBTX.

*Control current was significantly higher \((P<.05)\) than the residual current after IBTX at the indicated membrane potential. †The component of IBTX-sensitive current was significantly greater \((P<.05)\) in SHR than in WKY cells at the indicated membrane potential.

Figure 1. A and B, Whole-cell \( K^+ \) currents in WKY and SHR cerebral smooth muscle cells. Currents were elicited by incremental 10-mV depolarizing steps from −60 to 0 mV. IBTX (100 nmol/L) blocked a small component of outward current in the WKY cell, whereas a large current component was inhibited by IBTX in the SHR cell. C and D, Current-voltage relationships showing the effect of 100 nmol/L IBTX on peak macroscopic \( K^+ \) current in 18 WKY and 21 SHR cells, respectively. Shaded areas depict the current component sensitive to block by IBTX.

Statistics

All data are expressed as mean±SEM. Statistical comparisons between groups were made with one-way repeated-measures ANOVA with a subsequent Newman-Keuls test. Significance was accepted at a value of *P*<.05.

**Figure 2.** Scatterplot showing the peak membrane density of IBTX-sensitive \( K_{Ca} \) current (elicited at 0 mV) in WKY and SHR cerebral arterial muscle cells. Density is plotted as a function of cell capacitance. Each symbol represents a single WKY or SHR cell. The average peak density of IBTX-sensitive current was 0.59±0.08 pA/pF in WKY cells and 2.8±0.5 pA/pF in SHR cells, showing a 4.7-fold increase in SHR cells. Membrane capacitance for WKY and SHR cells showed similar values of 16.3±0.6 and 15.1±0.7 pF, respectively.

**Results**

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In Fig 1, panels A and B show macroscopic outward currents in WKY and SHR cells, respectively, that were generated by incremental 10-mV depolarizing steps from −60 to 0 mV. Outward currents were eliminated after equimolar replacement of \( K^+ \) by Cs⁺ in the pipette dialysate, identifying \( K^+ \) as the likely charge-carrying ion \((n=4, \text{not shown})\). The peak membrane density of depolarization-induced \( K_{Ca} \) current, defined as the outward current sensitive to block by 100 nmol/L IBTX, was compared in WKY and SHR cerebral arterial muscle cells. Currents recorded from WKY cells showed a small but consistent component of current inhibited by 100 nmol/L IBTX (Fig 1A). In contrast, noisier current traces showing a large component of current sensitive to block by IBTX predominated in SHR cells (Fig 1B). The averaged data in Figs 1C and 1D show that 100 nmol/L IBTX reduced maximum current density calculated at 0 mV by 21±3% in WKY (n=18) and by 65±11% in SHR (n=21) vascular smooth muscle cells. The IBTX-sensitive current corresponding to the \( K_{Ca} \) current component (shaded area) was the predominant contributor to voltage-elicited outward current in SHR but not WKY vascular smooth muscle cells at negative potentials and showed a significantly higher membrane density in SHR cells at potentials positive to −50 mV.

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Comparison of Single-Channel Properties of K<sub>Ca</sub> Channels

Subsequent experiments examined the possibility that altered single-channel properties accounted for the amplified K<sub>Ca</sub> current in cerebrovascular smooth muscle membranes of SHR. Fig 3A shows that the unitary amplitudes of single-channel currents obtained at six membrane potentials between −40 and +80 mV were similar for inside-out patches from WKY and SHR smooth muscle membranes. The resulting current-voltage relationship in Fig 3B, generated by plotting unitary current amplitude as a function of membrane potential, indicated single-channel conductances of 241 pS (WKY) and 235 pS (SHR). The recordings in Figs 3C and 3D illustrate that inside-out membrane patches from SHR and WKY patches, providing no evidence for altered voltage or Ca<sup>2+</sup> sensitivities for K<sub>Ca</sub> channels from WKY and SHR rats. Increasing the level of [Ca<sup>2+</sup>]<sub>i</sub>, resulted in a leftward shift of the Boltzmann relation on the voltage axis, which was not different between SHR and WKY rats. This effect was more pronounced at lower [Ca<sup>2+</sup>]<sub>i</sub>, levels, a finding previously reported by others. 20,21 Calculated values for V<sub>1/2</sub> at the physiological levels of [Ca<sup>2+</sup>]<sub>i</sub>, at 100 and 300 nmol/L, averaged 113 ± 1 and 68 ± 1 mV, respectively, in membrane patches from WKY rats. Similar values for V<sub>1/2</sub> at 106 ± 2 and 66 ± 2 mV were obtained at the same levels of [Ca<sup>2+</sup>]<sub>i</sub>, for K<sub>Ca</sub> channels in SHR membrane patches. Increasing [Ca<sup>2+</sup>]<sub>i</sub>, further to 1 μmol/L, appeared to result in an initial sudden increase in NPO, but steady-state NPO was not as markedly enhanced. Hence, V<sub>1/2</sub> values at 1 μmol/L [Ca<sup>2+</sup>]<sub>i</sub>, were 58 ± 1 and 56 ± 1 mV for K<sub>Ca</sub> channels in WKY and SHR patches, respectively. Thus, values of K and V<sub>1/2</sub> were similar under identical conditions of voltage and [Ca<sup>2+</sup>]<sub>i</sub>, for K<sub>Ca</sub> channels in WKY and SHR patches, providing no evidence for altered voltage or Ca<sup>2+</sup> sensitivity of K<sub>Ca</sub> channels during chronic hypertension.

Comparison of K<sub>Ca</sub> Channel α-subunit Expression

In initial control studies using cerebral arterial muscle membranes from WKY rats, immunoblotting reactions were performed to verify the specificity of the primary antibody, anti-α<sub>d13–926</sub> (AB in Fig 5), for its recognition site on the α-subunit of the K<sub>Ca</sub> channel. The left two lanes of the first immunoblot in Fig 5 (top) show that the K<sub>Ca</sub> channel α-subunit, which represents an immunoreactive protein with an apparent molecular mass of 125 kD, 16 was readily detected in lanes containing WKY membranes. The right two lanes demonstrate that this immunoreactive band was abolished by coincubation with anti-α<sub>d13–926</sub> with 1 μmol/L of the antigenic competing peptide (AB+CP in Fig 5), confirming the specificity of anti-α<sub>d13–926</sub> for its recognition sequence on the α-subunit of the K<sub>Ca</sub> channel. In the same lanes, hybridization
with the monoclonal antibody for the 42-kDa protein, β-actin, showed a similar signal density for this internal standard, demonstrating uniformity of lane loading.

Subsequently, the anti-α913-926 antibody was used to compare the expression levels of KCa channel α-subunit between cerebral arterial smooth muscle membranes of SHR and WKY rats. The second immunoblot in Fig 5 (bottom), which shows two adjacent lanes loaded with WKY (left) or SHR (right) membranes, illustrates that the density of the 125-kD immunoreactive band was higher in membranes from SHR. Data averaged from seven separate comparisons using cerebral arterial smooth muscle membranes of SHR and WKY rats indicated that the density of the 125-kD immunoreactive band (expressed as percentage of the β-actin signal) was 5.31 ± 1.69% for WKY rats and 21.97 ± 6.43% for SHR, suggesting a 4.1-fold increase in KCa channel α-subunit expression in SHR cerebral vascular smooth muscle membranes. The density signal of the β-actin internal standard was not different between SHR and WKY preparations.

Figure 5. Top, Immunoblot of the KCa channel α-subunit in cerebral smooth muscle membranes. Each lane was loaded with 20 μL of supernatant from cerebral arteries of WKY rats. The α-subunit was recognized by the antibody (AB) as a 125-kD diffuse band in lanes 1 and 2. No band was detected in lanes 3 and 4, when the antibody was coincubated with the competing antigenic peptide (AB+CP). The CP concentration was 1 μmol/L. β-actin, used as an internal standard, was detected as a 42-kD band in all four lanes. Bottom, Comparison of the expression of the KCa channel α-subunit in cerebral arterial muscle membranes from WKY rats (lanes 1 and 2) and SHR (lanes 3 and 4). Each lane was loaded with 20 μL of supernatant containing cerebral arterial muscle membranes. The density of the 125-kD immunoreactive band, corresponding to the known molecular size of the α-subunit, was greater in SHR than in WKY membranes. β-actin, used as an internal standard, showed similar expression between lanes.

Effect of IBTX on the In Situ Diameters of WKY and SHR Cerebral Arterioles

The functional role of the KCa channel in regulating the resting diameters of in situ SHR and WKY rat pial arterioles was evaluated in dura-open and dura-intact cranial windows. The resting diameters of pial arterioles on the cortical surface of WKY rats averaged 22 ± 1 and 27 ± 3 μm in dura-open and dura-intact windows, respectively, showing no significant difference. In the same preparations, resting diameters in SHR arterioles averaged 22 ± 1 and 24 ± 1 μm, respectively, dimensions similar to the arterioles of the normotensive animals.

In Fig 6, panels A and B demonstrate the effect of IBTX (0.1 to 100 nmol/L) on the diameters of in situ pial arterioles in dura-open and dura-intact cranial windows, respectively. In both preparations, concentration-dependent block of KCa channels by IBTX progressively constricted WKY and SHR pial arterioles. This constriction was significantly potentiated in SHR compared with WKY vessels at higher drug concentrations. Fig 6A shows that the diameter reduction in response to the maximal concentration of 100 nmol/L IBTX was 11 ± 2% in WKY arterioles compared with 33 ± 5% in SHR arterioles observed in dura-open cranial windows (n = 6 rats). Fig 6B shows similar, although accentuated, reactivity responses of WKY and SHR arterioles in dura-intact cranial windows, which constricted 39 ± 3% and 63 ± 11% in response to 100 nmol/L IBTX, respectively (n = 8 rats). Some SHR arterioles constricted severely even during infusion of lower IBTX concentrations associated with partial KCa channel inhibition. Fig 7 shows the reactivity responses to 10 nmol/L IBTX in a WKY arteriole (left frames) and two SHR arterioles (right frames) monitored through dura-intact cranial windows. Partial block of KCa channels by 10 nmol/L IBTX decreased the diameter of the WKY arteriole by 12%, whereas SHR arterioles were constricted by 64%. At 100 nmol/L IBTX, the same SHR arterioles were constricted so severely that their image was less than the absolute resolution...
of the video microscope system (±0.7 μm). Similar profound constrictions, which we interpreted as vasospasm, were observed in two other SHR preparations. The level of resting diameter and the concentration-dependent constrictions to IBTX in SHR arterioles were not altered by 1 μmol/L tetrodotoxin (n=4), implying that the diameter reductions induced by IBTX resulted from a direct block of KCa channels in cerebrovascular smooth muscle membranes and were not mediated indirectly by a change in neuronal activity and consequent release of vasoconstrictor neurotransmitters from the underlying cerebral cortex.22

**Discussion**

The present study demonstrates for the first time that the expression profile of K+ channels in the cerebral microcirculation may be a function of cardiovascular disease. This finding may have profound implications for the design of drug therapies, because identifying disease-specific K+ channel expression may permit the targeting of vasodilator drugs to those ion channels that are highly expressed in different cardiovascular pathologies. Our specific new findings show that (1) membrane KCa channel current is enhanced during chronic hypertension in cerebral resistance arteries, lending physiological relevance to this phenomenon only observed earlier in larger conduit vessels;6–11,23,24 (2) the expression level of the cerebrovascular KCa channel α-subunit is proportionally related to in situ blood pressure levels, suggesting that it represents a fundamental adaptive mechanism that permits cerebral arteries to buffer arterial excitability during chronic hypertension; and (3) although the KCa channel has multiple α-subunit isoforms that represent different channel phenotypes,24–28 detailed single-channel analyses in the present study indicate that this molecular mechanism for adaptation is not activated in cerebral resistance arteries exposed to chronic hypertension.

**KCa Current Is Increased in the Cerebral Microcirculation of SHR**

Initial reports of an enhanced K+ turnover in aortas from hypertensive rats has led to the proposal that the high-conductance KCa channel is a target of blood pressure modulation in large conduit arteries.6 In the present study, using a multifaceted approach of patch-clamp, Western, and in situ vascular reactivity methods, we have provided the first comprehensive report on the relationship between high blood pressure and KCa channel expression, phenotype, and physiological impact in the cerebral microcirculation. This microcirculation, unlike conduit arteries, represents a highly dynamic vascular bed where changes in the ionic mechanisms that regulate vascular tone profoundly impact regional tissue perfusion and cortical function. Hence, the initial finding of the present study, ie, that SHR cerebrovascular smooth muscle membranes show an amplified KCa channel current during exposure to chronic hypertension, is likely to be of extreme importance in understanding the cellular protective mechanisms that interact to set the level of cerebral tone during hypertensive disorders.

**Molecular Mechanism for Increased Cerebrovascular KCa Current in SHR**

The mechanism for the increase in total membrane current (ΔI) must represent changes in its factors (N·i·P0, where N is channel number, i is unitary current amplitude, and P0 is channel open-state probability). Hence, N, i, and P0 represent the three distinct sites of abnormality that could contribute to the elevated KCa current in cerebrovascular smooth muscle cells from SHR. Using a site-directed antibody directed against the S9/S10 linker of the KCa channel,16 we report a 4.1-fold increase in the expression (N) of the pore-forming α-subunit in SHR cerebrovascular smooth muscle membranes. This finding provides the first evidence that ion channel expression is abnormal in the cerebral circulation during chronic hypertension and further implies that the expression level of the pore-forming KCa channel subunit is a function of the blood pressure profile of the host animal.

Several molecular mechanisms are known to modify the voltage or Ca2+ sensitivity of expressed KCa channels, including the coupling of the α-subunit to its stimulatory β-subunit and the generation of alternatively spliced α-subunit isoforms.24–28 In this respect, an increased Ca2+ sensitivity has been suggested as a potential mechanism for the increased P0 of KCa channels in aortic muscle membranes of SHR.8 However, recent evidence suggests that the functional profile and regulation of KCa channels in the microcirculation do not resemble those of KCa channels in large vessels; hence, findings from conduit vessels cannot be readily extrapolated to resistance arteries.29,30 Furthermore, at present, the phenotypic profile of KCa channels exposed to chronic hypertension has not been fully defined by complete Boltzmann analysis in any vascular tissue. In the present study, single-channel slope conductance, which is a function of unitary current amplitude (i), was similar between KCa channels from WKY and SHR membrane patches. Furthermore, activation curves fit by a Boltzmann function revealed similar values of K and V1/2 for KCa channels from SHR and WKY rats. Hence, changes in
channel voltage or Ca$^{2+}$ sensitivity, which represent the two main determinants of $P_o$, were not detected. Thus, changes in the expression of the $K_{Ca}$ channel $\alpha$-subunit, rather than the functional properties of single $K_{Ca}$ channels, appears to represent the fundamental molecular mechanism that permits smooth muscle cells of the cerebral microcirculation to upregulate $K_{Ca}$ current during chronic hypertension. Identifying the initiating factors that permit this channel induction in the cerebral microcirculation, including events related to gene transcription and translation and channel subunit translocation and turnover at the membrane, may permit a fuller understanding of the process by which the blood pressure profile of the host animal dynamically influences the functional contribution of $K_{Ca}$ channels to cerebrovascular tone.\textsuperscript{23,24}

**$K_{Ca}$ Channels in Cerebrovascular Membranes May Be Unique**

Our comparison of unitary $K_{Ca}$ currents between membrane patches from SHR and WKY rats revealed one interesting feature of this channel common to both rat strains. Although the slope factor, $K$, an indicator of voltage sensitivity, was within the normal range of 9 to 17 often reported for $K_{Ca}$ channels in vascular muscle cells,\textsuperscript{19,25–27} the $V_{1/2}$ values of $+58$ mV (WKY) and $+56$ mV (SHR) calculated at 1 $\mu$mol/L [Ca$^{2+}$], were more positive than expected for $K_{Ca}$ channels in vascular muscle membranes. At the same level of [Ca$^{2+}$], $V_{1/2}$ values of $-12$ mV have been reported for rabbit aorta, whereas bovine mesenteric and coronary arteries show several $K_{Ca}$ channel isoforms with $V_{1/2}$ values ranging between $-60$ and $-41$ mV.\textsuperscript{19,25,26} High-conductance $K_{Ca}$ channels of rat aorta have also been reported to be highly activated by internal Ca$^{2+}$ (see Reference 8) compared with the $K_{Ca}$ channels of the rat cerebrovascular smooth muscle membranes of the present study, suggesting that the $\alpha$-subunit isoform, or its level of association with its regulatory $\beta$-subunit, differs between conduit vessels and the cerebral microcirculation. As reviewed by Carl et al.,\textsuperscript{19} this finding indicates either a low Ca$^{2+}$ sensitivity or a lower Ca$^{2+}$ set point of $K_{Ca}$ channels in the rat cerebral circulation compared with other vascular $K_{Ca}$ channel types that have been studied, a finding also suggested by Wang and Mathers\textsuperscript{31} in the only other detailed analysis of $K_{Ca}$ channel activation properties in the cerebral circulation. Although this lower Ca$^{2+}$ sensitivity would tend to dampen the contribution of $K_{Ca}$ channels to membrane potential, it suggests that a unique tissue-specific $\alpha$-subunit or closely associated regulatory protein may exist in the cerebral circulation, which may provide a novel target for physiological regulation and vasoactive drug binding.

**Physiological Impact of Cerebrovascular $K_{Ca}$ Channels During Hypertension**

Brayden and Nelson\textsuperscript{1} first demonstrated that $K_{Ca}$ channels act as homeostatic pathways to limit acute pressure-induced constriction of cerebral resistance arteries. In their study, IBTX-induced block of vascular $K_{Ca}$ channels triggered depolarization and constriction of pressurized rabbit cerebral arteries. A similar regulatory role for this channel has been proposed by Gokina et al.,\textsuperscript{5} who demonstrated that charybdotoxin strongly depolarized and constricted small human pial vessels mounted for myograph recording and enhanced action potential generation. However, the extent to which $K_{Ca}$ channels contribute to the resting tone of cerebral arteries in vivo still requires clarification, and preparation differences may exist. For example, in the cranial window preparation with the dura opened, the resting diameters of in situ rabbit and cat cerebral arteries are unaffected by pharmacological $K_{Ca}$ channel blockers.\textsuperscript{32,33} Under similar conditions, in situ rat pial and basilar arteries show small constrictions.\textsuperscript{34–36}

Importantly, a recent report by Paterno et al.\textsuperscript{35} has shown an increased functional contribution of $K_{Ca}$ channels to the resting tone of basilar arteries observed in dura-open cranial windows of stroke-prone SHR. In the same study, an increased $K_{Ca}$ channel contribution was also noted in basilar arteries from WKY rats, in which blood pressure was elevated by treatment with a nitric oxide synthase inhibitor.\textsuperscript{35} Our findings in smaller pial arterioles in the cerebral microcirculation monitored in both dura-open and dura-intact cranial windows substantiate that report and clearly illustrate that in situ cerebral arterioles of SHR profoundly rely on IBTX-sensitive $K_{Ca}$ channels to maintain resting diameter and oppose vascular tone during hypertension. In addition, two other findings of the present study may be important. First, pial arterioles from SHR and WKY rats showed similar resting diameters, implying that the upregulation of $K_{Ca}$ channels during hypertension does not produce active dilation but, instead, nullifies an increased tendency of the cerebral resistance vessels to constrict. The vasospasm we observed in some SHR pial arterioles when $K_{Ca}$ channels were pharmacologically blocked by IBTX attests to the fundamental role that $K_{Ca}$ channel overexpression plays in maintaining the normal resting diameter of cerebral arterioles exposed to chronic hypertension. Second, pial arterioles from both SHR and WKY rats showed a stronger constrictor response to IBTX in dura-intact compared with dura-open cranial windows, suggesting a higher contribution of the $K_{Ca}$ channel current to resting tone in the intact preparation. Although it is possible that experimental damage accounts for this activity difference, this finding also raises the possibility that the dura mater per se may be an unrecognized modulator of vascular $K_{Ca}$ channel activity in cerebral arterioles. Notably, the dura is sometimes regarded as functionally inert, but it is equipped with nerve terminals containing 5-hydroxytryptamine, acetylcholine, and other vasoactive substances, which may contact the walls of cerebral vessels.\textsuperscript{36,37} Underlying native factors that regulate resting tone may profoundly influence the reactivity of in situ cerebral vessels\textsuperscript{36} and, hence, also may potentially influence the resting $P_o$ of $K_{Ca}$ channels.

**Pressure-Induced Upregulation May Be Specific to the $K_{Ca}$ Channel**

The findings of the present study raise the intriguing possibility that the population of K$^+$ channels that regulate vascular excitability in the cerebral circulation may be altered during cardiovascular pathologies. Furthermore, defining the K$^+$ channel profile in cerebral arterial smooth muscle mem-
branes during different disease states may permit the development of vasoactive drugs targeted to highly expressed and functionally relevant K+ channel types. However, because the resting level of K+ efflux across cerebrovascular smooth muscle membranes represents the product of the expression, unitary conductances, and open-state probabilities (N • i • Po) of all K+ channel types, it is possible that the expression of at least several kinds of K+ channels may be modified during chronic hypertension. Delayed and inward-rectifying K+ channels have been postulated to contribute to the regulation of cerebrovascular tone, and glibenclamide-sensitive K+ channels also may modulate cerebrovascular excitability under some conditions.10-45

To date, however, early studies in the SHR vasculature have not documented an enhanced K+ current through other types of K+ channels, which are insensitive to Ca2+-activation. Rather, Martens and Gelband4 recently have reported a reduced density of delayed-rectifier K+ current in renal arteries of SHR, and Kitazono et al45 observed an impaired vasodilator function of glibenclamide-sensitive K+ channels in basilar arteries of stroke-prone SHR. Considering these findings, it appears that the increased expression of KCa channel α-subunits in cerebral arterial muscle membranes of hypertensive rats may represent a unique property of the KCa channel type. The selective upregulation of this channel may provide a novel target for vasodilator therapy during chronic hypertension, while concurrently providing a crucial homeostatic mechanism to preserve normal levels of cerebral blood flow.

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References


Increased Expression of Ca\textsuperscript{2+}-Sensitive K\textsuperscript{+} Channels in the Cerebral Microcirculation of Genetically Hypertensive Rats: Evidence for Their Protection Against Cerebral Vasospasm

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