Slowly Inactivating Potassium Conductance (I_d)  
A Potential Target for Stroke Therapy

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Background and Purpose—Excessive accumulation of extracellular glutamate results in the death of most, but not all, neurons in the central nervous system. Understanding the unique properties of cells that can withstand this excitotoxic challenge may identify specific targets for novel stroke therapies.

Methods—A combination of in vivo methods for analysis of excitotoxic cell death after activation of N-methyl-D-aspartate (NMDA) receptors and in vitro patch-clamp analysis of specific conductances in hypothalamic slices and dissociated cells has been used to assess the roles of specific potassium conductances in delayed cell death after NMDA receptor activation.

Results—We report that a specific D-type potassium conductance (I_d), necessary for the rapid repolarization of the membrane after a strong depolarization, serves such a protective purpose in magnocellular neurons of the paraventricular nucleus. Manipulations that inhibit this current (4-aminopyridine or angiotensin II) increase neuronal excitability and augment cell death after NMDA receptor activation. In addition, this protection is not observed in magnocellular neurons of spontaneously hypertensive rats, and intriguingly it can be reestablished by blocking angiotensin II receptors in these animals.

Conclusions—These observations provide a persuasive experimental explanation for the unexpected finding that therapeutic treatments for hypertension that block central as well as peripheral angiotensin type 1 receptors reduce the severity and occurrence of stroke.  

Key Words: angiotensins ■ excitotoxicity ■ neuroprotection ■ N-methyl-D-aspartate ■ potassium channels ■ stroke, experimental ■ stroke prevention ■ rats

Sustained exposure to glutamate1 or other excitatory amino acids2 destroys central nervous system (CNS) neurons. This “excitotoxicity,” which is likely initiated by the binding of glutamate to the N-methyl-D-aspartate (NMDA)3 receptor followed by a subsequent influx of calcium,4 may be responsible for the cell death observed in degenerative, traumatic, and vascular disorders of the CNS.5,6 Not all CNS neurons, however, are uniformly sensitive to excitotoxicity. Autonomic motor neurons, for example, are resistant to the degenerative effects of amyotrophic lateral sclerosis, which can be mimicked by exogenous application of NMDA, while neighboring somatic neurons are not.7 A similar disparity in cell viability after application of ibotenic acid, a glutamate analogue, has been noted between neuronal subtypes in the hypothalamic paraventricular nucleus (PVN).8 One of these groups, the magnocellular neurons of the PVN, displays a remarkable lack of sensitivity to ibotenate even though the neighboring parvocellular neurons are destroyed.8 One clue as to the possible reason for this survival may be gleaned from the observation that these cells exhibit a conservative electrophysiological response when tested with an exogenous NMDA receptor agonist [dl-(tetrazol-5-yl)glycine] (NMDAa).9 By contrast, the parvocellular neurons exhibit robust, long-lasting depolarizations when challenged with the identical dose of NMDAa. This raises the possibility that the initial response of a cell to glutamate may be a strong indicator of its prospects for survival.10

A strong case can be made for the role of the postsynaptic depolarization in cell death.11,12 For example, after focal ischemia, the development of damage in the penumbra has been linked to the frequency of these depolarizations.13,14 Although mediated primarily by the activation of NMDA receptors after the release of glutamate from the energetically compromised core of the lesion,12 it seems that it is the depolarization itself15 and not the activation of postsynaptic NMDA receptors per se that is important in increasing infarct size. These depolarizations can be mimicked in a number of in vitro preparations by exogenous application of glutamate, and as is the case during focal ischemia, a preponderance for extended neuronal depolarization in response to glutamate is strongly indicative of subsequent death in neurons.16 This raises the intriguing possibility that one way to protect cells...
from excitotoxic cell death would be to prevent the initial depolarization. We have demonstrated that the ability of a cell to generate an extended neuronal depolarization is inversely related to both the amplitude and duration of the hyperpolarizing afterpotential that follows an action potential.9 Since magnocellular neurons in PVN fail to generate these plateau-like depolarizations, we hypothesized that their ability to regulate their membrane potential when challenged with accumulating levels of glutamate may confer resistance to these cells during an excitotoxic challenge.

In the present study we focus on a D-type potassium conductance (ID) that slows membrane depolarization and may serve to dampen excitatory inputs.17,18 The relatively small depolarization in the magnocellular neurons can be augmented profoundly by 4-aminopyridine (4-AP)9 at doses that inhibit the ID selectively.17,19 This observation raises the possibility that this intrinsic voltage-gated conductance, by regulating the excitability of magnocellular neurons, may serve a protective function.

The present study expands on this observation by biophysically and pharmacologically characterizing ID in magnocellular PVN neurons. We also demonstrate that a micromolar dose of 4-AP not only accentuates and prolongs the depolarization of these cells in response to NMDAA but also causes a general increase in postsynaptic membrane excitability. The former effect may result from the temporal summation of putative dendritic calcium spikes, which in the absence of the delay current are now free to invade the soma. The manipulation of the D current in vivo also has the predicted effects on cell viability. Inhibition of ID and the subsequent application of NMDAA destroy previously resistant magnocellular neurons. Finally, a tonic inhibition of this conductance by angiotensin II (Ang II) may contribute to the increased cell damage in spontaneously hypertensive rats (SHR). This cell damage can be prevented by blockade of Ang II receptors, suggesting that compounds that can activate ID in neurons may serve a neuroprotective role.

Materials and Methods

Experimental Protocol

Experiments were performed on male Sprague-Dawley rats (weight, 150 to 525 g; Charles River, Quebec, Canada), and were performed in accordance with the guidelines of the Canadian Council for Animal Care. The animals were anesthetized with sodium pentobarbital (65 mg/kg IP) and placed in a stereotaxic frame; the skull was exposed, and a small burr hole was drilled in the skull such that a cannula electrode (tip diameter, 150 μm) could be advanced into the region of the PVN according to the coordinates of Paxinos and Watson (−0.9 mm bregma, 0.5 mm lateral, 7.5 mm ventral).

Each animal received a 1.0-μL (2 × 0.5 μL) microinjection to each PVN according to 1 of the following 7 protocols: saline/saline, saline/NMDA, 4-AP/saline, 4-AP/NMDA, Ang II/saline, Ang II/NMDA, and saralasin/NMDA. The incision was then closed, and the animal received the analgesic buprenorphine (0.03 mg/kg SQ) to aid postoperative recovery. Animals were allowed to recover for 3 days, after which they were overdosed with sodium pentobarbital (100 mg/kg) and perfused with 0.9% saline followed by 10% formalin through the left ventricle of the heart. The brain was removed and placed in formalin overnight at 4°C. The brain was cut into a smaller block containing PVN and stored in a 30% sucrose, 0.1 mol/L phosphate buffer at 4°C for at least 2 days.

Figure 1. PVN neurons are differentially susceptible to NMDA-induced cell death. a. Histological cresyl violet–stained coronal section through the PVN (bar=100 μm) after microinjection of NMDA or vehicle control. Cell loss is observed in the parvocellular neurons 3 days later, while magnocellular neurons do not exhibit any gross morphological changes that might be indicative of cell death. The contralateral PVN shows lack of effect of NMDAa or vehicle control. b, Cell death for the magnocellular and parvocellular regions in 1 animal is quantified by counting surviving neurons in 11 consecutive histological sections through PVN (section thickness=20 μm). Red solid bars indicate cell numbers in vehicle-injected controls, and striped blue bars indicate cell numbers in NMDAa injection sites. c, Mean total number of surviving neurons in the magnocellular (magn) and parvocellular (parvo) regions after either saline (red) or NMDAa (blue) application. No change was observed in the magnocellular region (control, 2546 ± 24; NMDAa, 1981 ± 38; ***P < 0.001).

Histology

The blocks were mounted, covered with Tissue-Tek O.C.T. compound, and flash-frozen in 2-methylbutane (cooled by dry ice) for 45 seconds. With the use of the Frigocut 280, 20-μm coronal sections were cut through the area of PVN. These sections were mounted and stained with cresyl violet. The histological locations of the microinjection sites were verified at the light microscope level by an observer unaware of the experimental conditions. Only those animals with microinjection sites within the boundaries of PVN were analyzed further.

Magnocellular neurons were differentiated from parvocellular neurons and other cellular material by specific morphological characteristics.20 In addition to the anatomic location of the neuron...
within PVN, morphological size was used to further characterize neuronal type. Neurons with soma diameter of 20 to 25 μm and intact nuclei were characterized as viable magnocellular neurons. Neurons with soma diameter of 10 to 15 μm and intact nuclei were characterized as viable parvocellular neurons. Neurons with soma diameter of between 15 and 20 μm were not included in the study because they could not be reliably classified as belonging to either subpopulation. Histological sections were viewed under high magnification (×40) at the light microscope level, and a grid was superimposed over each area of PVN. This superimposed grid was used to count magnocellular and parvocellular neurons. To prevent the double counting of neurons, a neuron that came to lie on a vertical grid line was deemed to belong to the grid to the immediate right, and a neuron that came to lie on a horizontal grid line was deemed to belong to the grid directly above it. Following this method, a sum of the sections was established for magnocellular and parvocellular neurons from each hemisphere of PVN. Comparative analysis was performed whereby neurons were counted in 20-μmol/L sections following the initial rostral emergence of PVN through to the caudal limits of the nucleus (10 or 11 sections). Cells were only counted if they showed well-differentiated Nissl staining, a normal nuclear membrane, and clear cytoplasmic structure. All counts given incorporate Abercrombie’s correction for double counting.21

Statistical Analysis
Comparisons were performed with paired or unpaired Student’s t test or by ANOVA followed by Newman-Keuls post hoc test.

**Figure 2.** Electrophysiological correlate of differential susceptibility in PVN. a, Whole-cell recordings illustrate the cellular response to application of 1 μmol/L NMDA α in coronal hypothalamic slices. Typical responses from magnocellular (top) and parvocellular (bottom) neurons are shown. Triangle indicates time at which a 2-second application of NMDAα was initiated. Bars=5 seconds, 15 mV. bi, Mean depolarization and spike frequency during the first second of the response to NMDAα application in magnocellular (red; n=21) and parvocellular (blue; n=11) neurons (**P<0.01). bii, Current-voltage (I-V) curve calculated from voltage ramps during bath application of NMDAα. There is no difference between the amount of current passed by NMDA receptors on magnocellular (blue; n=4) versus parvocellular (orange; n=4) neurons.

**Electrophysiology**
Male Sprague-Dawley rats (weight, 150 to 250 g; Charles River, Quebec, Canada) were killed by decapitation; the brain was removed quickly from the skull and immersed in cold (1°C to 4°C) artificial cerebrospinal fluid (aCSF). The brain was blocked, and 400-μm hypothalamic slices, which included the PVN, were prepared as described previously.22 Slices to be used for blind patch recordings were incubated in oxygenated aCSF (95% O₂/5% CO₂) for at least 90 minutes at room temperature. Twenty minutes before recordings were made, the slice was transferred into a modified interface-type recording chamber and continuously perfused with aCSF at a rate of 1 mL/min. Alternatively, for the preparation of dissociated neurons, the PVN was microdissected out of these slices and placed in Ca²⁺- and Mg²⁺-free aCSF with 1.5% trypsin at 37°C. Cells were gently trititated at 10-minute intervals until dissociated from connective tissue. Cells were then washed, spun, resuspended in aCSF, and plated in 35-mm plastic petri dishes. These dishes were then placed within a 5% CO₂ environment at 37°C for 1 hour until the cells attached to the dish.23 The petri dishes were then filled with either Dulbecco’s minimum essential medium or Neurobasal A medium (Gibco). Both media contained antibiotics (100 U/mL penicillin/streptomycin); Neurobasal A was additionally supplemented with 0.5 mmol/L L-glutamine. Cells were maintained in this environment before voltage clamp recordings were obtained by means of whole-cell patch techniques from cells in these petri dishes perfused with aCSF at a rate of 1 mL/min. Magnocellular neurons (>15 μm in diameter) were selected for recording on the basis of their size, and their identity was confirmed by the presence of a large I₅.
Whole-cell recordings were obtained with the use of pipettes (resistance of 4 to 6 MΩ) filled with a solution containing the following (in mmol/L): potassium gluconate 140, CsCl 0.1, MgCl₂ 2, EGTA 1.1, HEPES 10, Na₂ATP 2, adjusted at pH 7.25 with KOH. The standard bath solution contained the following (in mmol/L): NaCl 140, KCl 5, MgCl₂ 1, CaCl₂ 2, HEPES 10, glucose 10, tetrodotoxin 1 μmol/L. Signals were amplified, collected, and processed with the use of an Axopatch 200B (Axon Instruments) amplifier, a 1401plus analog-digital interface, and Signal software from CED.

Reagents

For in vivo experiments, the high-affinity agonist for the NMDA receptor dl-(tetrazol-5-yl) glycine (Colour Your Enzyme) was microinjected into PVN at the concentration of 1 μmol/L per microliter. The nonselective K⁺ channel blocker 4-AP was microinjected into PVN at the concentration of 100 nmol/L per microliter. All drugs were dissolved in saline. For slice electrophysiology experiments, the NMDA agonist was prepared as a 1-mmol/L stock solution in aCSF and refrigerated until required. This agonist has greater affinity for the receptor than NMDA, with little affinity for other glutamate receptors, and does not rely on the extracellular availability of glycine to activate the receptor.²⁴ The agonist was bath-applied at the concentration (and for the duration) specified in the text and figure legends. 4-AP was prepared as needed and was administered at a concentration of 100 μmol/L. Ang II (Sigma) and α-dendrotoxin (Alomone) were freshly prepared on the day of the experiments.

Results

We microinjected the NMDAa into PVN and used quantitative histological techniques to compare numbers of surviving magnocellular and parvocellular neurons 3 days after treatment. While the mean number of surviving parvocellular neurons was significantly reduced by NMDAa (control, 2446±24; NMDAa, 1981±38; P<0.001), there was no significant reduction in magnocellular neuron numbers (control, 2098±37; NMDAa, 2056±49; P>0.05) (Figure 1). These observations correlate strongly with the electrophysiological response of these neurons to NMDA receptor activation in an acute brain slice preparation (Figure 2).⁹ Parvocellular neurons exhibit a rapid increase in firing frequency followed by a sustained depolarizing response to application of NMDAa (1 to 2 seconds). This previously classified long-duration plateau depolarization⁹ is similar to the extended neuronal depolarization described in hippocampal neurons.¹⁶ A causal relationship between these extended neuronal depolarizations and subsequent cell death is well established.¹⁶ This finding, as well as the hypothesis that a sustained depolarization in response to the initial glutamatergic insult is a necessary first step in the initiation of cell death,³,⁴ is further supported by the observation that excitotoxin-resistant magnocellular neurons of the PVN do not exhibit such rapid, sustained depolarizations in response to NMDA receptor activation (Figure 2). The remainder of this study was designed to elucidate the specific mechanisms underlying the unique ability of magnocellular neurons to survive excitotoxic challenge.

We considered the possibility that this dichotomy in responses to NMDAa may reflect a difference in NMDA receptor kinetics¹⁰ resulting from variations in the heteromeric assembly of receptor subunits.²⁵ Different receptor conformations can result in dramatic changes in the ability of the NMDA receptor to pass current²⁵ or even alter its selectivity for Ca²⁺ ions,²⁶ which initiate the cascade of events leading to cell death.⁴,²⁷,²⁸ Using voltage ramps (Figure 2bii), we found no appreciable difference between mag-
nocellular (n=4) and parvocellular (n=6) neurons either in the degree of Mg\(^{2+}\) block or in the amount of current passed at comparable membrane potentials in response to exogenous NMDAa.

We next tested the hypothesis that differences in the intrinsic membrane conductances in the 2 cell types may be responsible for the divergent responses to NMDAa. Intriguingly, one of the distinguishing features of magnocellular neurons is the dominant transient potassium conductance they express.\(^{29,30}\) The inhibition of this conductance speeds the return of the membrane potential to baseline after a hyperpolarizing pulse and also decreases the time to the first spike (Figure 3a). This translates into a net increase in postsynaptic excitability, with neurons exhibiting an increase in spiking in response to depolarizing current pulses when I\(_\text{D}\) is blocked by 100 \(\mu\text{mol/L}\) 4-AP (Figure 3b). In addition to this manipulation of I\(_\text{D}\) by an exogenous substance, we also demonstrate that Ang II, a peptide that has excitatory neurotransmitter actions in PVN,\(^{31–33}\) has effects similar to low-dose 4-AP in increasing the number of action potentials in response to depolarizing current pulses (Figure 3c). We have also obtained voltage clamp recordings from dissociated PVN neurons and observed the presence of a rapidly activating, slowly inactivating whole-cell current that is distinct from I\(_\text{A}\) (Figure 4a) and is sensitive to micromolar doses of 4-AP\(^{17}\) and submicromolar concentrations of \(\alpha\)-dendrotoxin\(^{17}\) (Figure 4b).

The potential importance of I\(_\text{D}\) in regulating the neuronal excitability of magnocellular neurons is highlighted by the observation that magnocellular neurons exhibit plateau-like depolarizations when challenged with NMDAa in the presence of 100 \(\mu\text{mol/L}\) 4-AP but not in the absence of this compound (Figure 5). We now demonstrate that the onset of the plateau is characterized by the broadening of successive spikes during the ramp-up depolarization (Figure 5a). This may result from the unmasking of presumptive dendritic Ca\(^{2+}\) spikes that can be generated in response to a depolarization of either the somatic membrane by a current pulse (Figure 5b) or by a depolarization of the dendrites by NMDA receptor activation.\(^{34}\)
The hypothesis that the shunting of excessive depolarization by \( I_D \) is important in conferring resistance to magnocellular neurons to excitotoxicity was examined by testing the ability of the cells to withstand excitotoxic challenge in vivo. In experiments evaluating cell death after microinjection of NMDAa into PVN with and without pretreatment by microinjection of 4-AP, we observed a statistically significant reduction in magnocellular neuron numbers in PVN pretreated with 4-AP (1618 ± 33) compared with NMDAa (2056 ± 49; \( P < 0.01 \)) or Ang II (2043 ± 51; \( P < 0.01 \)) alone (Figure 7b). These data further support the conclusion that the dominant role played by this 4-AP– and Ang II– sensitive potassium conductance in controlling the excitability of PVN magnocellular neurons is also responsible for the resistance of these neurons to excitotoxic cell death.

Intriguingly, one of the primary risk factors for stroke is hypertension, a condition that is normally associated with increased circulating and central levels of Ang II. The aforementioned observations therefore suggest a mechanism through which such increased levels of Ang II may exacerbate stroke-induced cell death. This suggestion is supported by reports that hypertensive treatments based on the blockade of Ang II receptors have dramatic effects in prolonging life expectancy that cannot be explained simply by their blood pressure–lowering effects. The blockade of Ang II receptors also decreases the frequency and severity of stroke in a variety of animal models at doses that have no effect on blood pressure. This hypothesis would predict that in hypertensive rats with increased central Ang II, the magnocellular neurons should lose their resistance to excitotoxins. We have tested this hypothesis in SHR by again microinjecting NMDAa or vehicle control into PVN and counting surviving neurons 3 days later. After this treatment, we observed a loss of parvocellular neurons (82.1 ± 2.2% surviving) similar to that found in normotensive animals, but the resistance of magnocellular neurons observed in normotensive animals was no longer present in SHR (control, 1565 ± 13; NMDAa, 1113 ± 45; \( P < 0.01 \)) (71.1 ± 4.9% surviving; Figure 8). To confirm that Ang II was responsible for this loss of resistance, NMDAa was microinjected into PVN of SHR immediately after the Ang II receptor antagonist saralasin. Under these conditions, magnocellular neurons were resistant to excitotoxic cell death, with no observed cell loss 3 days later (1446 ± 137; \( P > 0.05 \); Figure 8), while the parvocellular neurons were still significantly reduced in number (83.9 ± 2.0% surviving). These findings provide the first direct evidence that elevated Ang II concentrations in the CNS of hypertensive subjects may contribute to increased susceptibility for stroke and that these actions can be prevented by central Ang II receptor blockade.

**Discussion**

The observations presented here suggest that transiently activated \( K^+ \) conductances and, more specifically, the delay current regulate the excitability of magnocellular PVN neurons and prevent glutamate-mediated excitotoxic cell death. The importance of voltage-gated potassium conductances in regulating neuronal excitability as it relates to other neuronal functions (such as synaptic transmission and neuronal development) is well established, but their role in conferring resistance to excitotoxic cell death is interesting and novel, does it have physiological relevance in this system? To answer this question, we examined whether Ang II, a substrate that is endogenous to PVN and one that also increases neuronal excitability by inhibiting \( I_D \), would alter the profile of neuronal viability. Microinjection of Ang II into PVN before NMDA agonists eliminated the resistance to cell death normally observed in magnocellular neurons (Figure 7a). After this treatment, the number of surviving magnocellular neurons was significantly reduced (1693 ± 33) compared with NMDAa (2056 ± 49; \( P < 0.01 \)) or Ang II (2043 ± 51; \( P < 0.01 \)) alone (Figure 7b). These data further support the conclusion that the dominant role played by this 4-AP– and Ang II– sensitive potassium conductance in controlling the excitability of PVN magnocellular neurons is also responsible for the resistance of these neurons to excitotoxic cell death.
pathologies has been well documented. The present findings are unique in that instead of using the absence or dysfunction of a particular channel as evidence for its role in the expression of a pathology, they demonstrate how neurons may utilize repolarizing voltage-gated currents to protect themselves and thereby prevent the expression of a pathology. They are also consistent with the goals of current POST stroke trials examining whether enhancing potassium conductances serves a neuroprotective role.

Our results support the hypothesis that neuronal depolarizations are a critical first step in the excitotoxic pathway. Since these events are initiated by activation of NMDA receptors, we also investigated whether magnocellular neurons may possess NMDA receptors that exhibit a blunted response, as has been reported in other systems. We found no evidence of a smaller NMDA conductance in magnocellular neurons. Thus, the electrophysiological step that differs between the 2 cell types is the degree of membrane depolarization. This is consistent with findings that the exogenous induction of depolarizations in the penumbra after an ischemic event, even without the involvement of NMDA receptors, can increase the size of the infarct.

Our observations may also provide an important physiological corollary for the recent demonstration that K^+ channel interacting proteins can increase the conductance and inactivation time constant of Kv4.2 in a Ca^2+-dependent manner. These proteins may be activated in response to the surge in Ca^2+ that accompanies synaptic activation specifically, but not exclusively, when the NMDA receptor is involved. This would result in an increase in the conductance of K^+ channels and provide a safety valve that would protect the cell by preventing the passage of large depolarizations from the dendrites to the soma after intense synaptic activity.

Figure 6. Role of I_d in conferring neuroprotection. a, When coapplied with 4-AP, NMDAa kills similar numbers of both magnocellular (77.1 ± 6.4%; n = 7) and parvocellular (79.5 ± 2.2%; n = 7) neurons, as indicated in these cresyl violet–stained coronal sections (bar = 75 μm). b, Cell death for the magnocellular region in 1 animal is quantified by counting surviving neurons in 11 consecutive histological sections through PVN (section thickness = 20 μm). Red solid bars indicate cell numbers in vehicle-injected controls, and light blue bars indicate cell numbers in 4-AP + NMDAa injection sites. c, Mean total number of surviving magnocellular neurons after either saline (red; 2098 ± 37.5), NMDAa (blue; 2056 ± 49), 4-AP (red hatch; 2008 ± 29), or NMDAa preceded by 4-AP (light blue; 1618 ± 75; **P < 0.01 compared with NMDAa).
equally capable, in the presence of TTX, of generating dendritic Ca\textsuperscript{2+} spikes after NMDA receptor activation,\textsuperscript{34} these events are not normally observed in magnocellular neurons with functional Na\textsuperscript{+} channels. These data suggest a functional difference in expression of I\textsubscript{D} in magnocellular versus parvocellular neurons that is supported by the current clamp data. Although our inability to specifically identify parvocellular PVN neurons in culture, when combined with the reduced dendritic tree of such dissociated cells, precludes meaningful voltage clamp analysis of relative current density, additional supportive data for this concept come from recent voltage clamp studies reporting that 50\% of parvocellular neurons express transient potassium conductances that are 4-AP insensitive\textsuperscript{29} and thus must be concluded to lack I\textsubscript{D}.

The use of 4-AP raises questions about the specificity of the effects observed. A similar dose of 4-AP has been shown to selectively inhibit a slowly activating K\textsuperscript{+} current in spinal cord astrocytes,\textsuperscript{43} raising the possibility that the effects observed in the present study may be the result of a disruption of glial K\textsuperscript{+} homeostasis. Although we have not tested this possibility directly, the following indirect lines of evidence suggest that this scenario is not likely in our system. First, we observed no change in resting membrane potential of the magnocellular neurons after 4-AP application. Such a change might be expected if K\textsuperscript{+} buffering by glia were altered. Second, 4-AP by itself had no effect on the viability of magnocellular or parvocellular neurons in vivo, suggesting that any potential effects on glial K\textsuperscript{+} currents do not affect neuronal excitability in PVN. We also did not focus on the well-documented presynaptic effects of 4-AP. In other systems, low doses of 4-AP increase the spontaneous release of both excitatory\textsuperscript{44} and inhibitory\textsuperscript{45} neurotransmitters. We found no increase in spontaneous synaptic events in PVN neurons after 4-AP application, nor did we, as mentioned
above, see any changes in neuronal viability after 4-AP alone that may be indicative of an increase in glutamate release.

The present finding that I_{D} must be inhibited to observe these events in magnocellular neurons suggests that the role of this conductance may be to electrically uncouple the dendrites from the soma during a strong dendritic depolarization. Alternatively, this conductance may prevent somatic spikes from back propagating to the dendrites and initiating Ca^{2+} spikes, which may serve to relieve the Mg^{2+} block from NMDA receptors and further enhance the synaptic depolarization. By spatially restricting this depolarization, I_{D} may serve to protect the soma in the face of pathologically elevated levels of extracellular glutamate. Our findings are consistent with recent work demonstrating that 4-AP increases the likelihood of dendritic Ca^{2+} spike initiation in CA1 pyramidal cells and cerebellar Purkinje cells. We cannot rule out the possible and perhaps even important contributions of other subsets of K^{+} channels. In particular, the I_{A} is well suited to regulating excitability of CA1 pyramidal neurons. The fact that 4-AP and Ang II can both inhibit I_{A} as well as I_{D} does not permit us to rule out potential roles for I_{A} in contributing to the resistance of the magnocellular neurons. Interestingly, CA3 neurons are much more sensitive to kainite excitotoxicity than CA1 neurons, the latter of which also express a prominent I_{D}. While this would seem to validate our own conclusions about the importance of this conductance in protecting cells, we are hesitant to make such a comparison, especially in view of the conflicting data demonstrating that CA1 neurons are in fact more sensitive to hypoxia than CA3 neurons.

Although the precise molecular profile of channels that are responsible for the I_{D} is not yet known, it has been speculated, on the basis of their sensitivity to dendrotoxin, that members of the Shaker-related Kv1 family, primarily Kv1.1, Kv1.2,
and Kv1.6, may be involved.51 In addition to the fact that these subunits can be assembled in a variety of heteromers, additional complexity is conferred by the finding that the Kv1 subfamily can also associate with 1 of 2 β subunits that can further alter the kinetics of current inactivation. Small amounts of mRNA for these β subunits have been localized in the hypothalamus.52

Finally, the modulation of this delay current by Ang II, an endogenous messenger in this nucleus, provides the clearest evidence that this current may play an important physiological role in regulating excitotoxicity. Although the intracellular steps through which Ang II may inhibit I<sub>D</sub> are unclear, we speculate that it involves an interaction between the G protein–coupled angiotensin type 1 receptor and the subunits that constitute the channel. Recent work has demonstrated that I<sub>D</sub> can be modulated by another type of G protein–coupled receptor, the metabotropic glutamate receptors.53 The finding that blocking angiotensin type 1 receptors confers resistance to magnocellular neurons in SHR implicates Ang II as an essential factor in the increased stroke risk after hypertension.

We have shown here that modulation of this conductance by 4-AP or Ang II results in predictable effects on the response of these neurons to NMDA agonists. In contrast, the relative enhancement of the transient K<sup>+</sup> conductance, by inhibiting the actions of Ang II, may lower the probability and consequences of stroke-induced cell death. This neuronal interaction between postsynaptic K<sup>+</sup> conductances that regulate membrane excitability and glutamate may therefore represent a novel target for therapies directed toward reducing both the consequences of stroke.

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