Sodium Pump Activity, Not Glial Spatial Buffering, Clears Potassium After Epileptiform Activity Induced in the Dentate Gyrus

ZHI-QI XIONG AND JANET L. STRINGER
Department of Pharmacology and Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030

Xiong, Z.-Q. and J. L. Stringer. Sodium pump activity, not glial spatial buffering, clears potassium after epileptiform activity induced in the dentate gyrus. J. Neurophysiol. 83: 1443–1451, 2000. A number of mechanisms have been proposed to play a role in the regulation of activity-dependent variations in extracellular potassium concentration ([K\textsuperscript{+}]\textsubscript{o}). We tested possible regulatory mechanisms for [K\textsuperscript{+}]\textsubscript{o} during spontaneous recurrent epileptiform activity induced in the dentate gyrus of hippocampal slices from adult rats by perfusion with 8 mM potassium and 0-added calcium medium in an interface chamber. Local application of tetrodotoxin blocked local [K\textsuperscript{+}]\textsubscript{o} changes, suggesting that potassium is released and taken up locally. Perfusion with barium or cesium, blockers of the inward rectifying potassium channel, did not alter the baseline [K\textsuperscript{+}]\textsubscript{o}, the ceiling level of [K\textsuperscript{+}]\textsubscript{o}, reached during the burst, or the rate of [K\textsuperscript{+}]\textsubscript{o} recovery after termination of the bursts. Decreasing gap junctional conductance did not change the baseline [K\textsuperscript{+}]\textsubscript{o}, the half-time of recovery of the [K\textsuperscript{+}]\textsubscript{o}, after the bursts but did cause a decrease in the ceiling level of [K\textsuperscript{+}]\textsubscript{o}. Perfusion with furosemide, which will block cation/chloride cotransporters, or with low chloride did not change the baseline [K\textsuperscript{+}]\textsubscript{o}, the half-time of recovery of [K\textsuperscript{+}]\textsubscript{o}, after the bursts but did increase the ceiling level of [K\textsuperscript{+}]\textsubscript{o}. Bath or local application of ouabain, a Na\textsuperscript{+}/K\textsuperscript{+}-ATPase inhibitor, increased the baseline [K\textsuperscript{+}]\textsubscript{o}, slowed the rate of [K\textsuperscript{+}]\textsubscript{o} recovery, and induced spreading depression. These findings suggest that potassium redistribution by glia only plays a minor role in the regulation of [K\textsuperscript{+}]\textsubscript{o}, in this model appears to be uptake via a Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, most likely neuronal.

INTRODUCTION

Neuronal activity is associated with a rise in the extracellular potassium concentration ([K\textsuperscript{+}]\textsubscript{o}) caused by efflux of potassium during action potential repolarization. Neuronal activity, in the absence of clearance mechanisms, would cause the [K\textsuperscript{+}]\textsubscript{o}, to rise in seconds to values that would abolish all electrical activity (Coles 1986). However, it is known that during intense evoked neuronal activity or spontaneous epileptiform activity in the cortex (Heinemann and Lux 1977; Hotson et al. 1973; Prince et al. 1973) and hippocampus (Benninger et al. 1980; Knjvic et al. 1980) [K\textsuperscript{+}]\textsubscript{o}, rises to a ceiling level of 10–12 mM from a resting level of 3 mM. The occurrence of a plateau, or ceiling, level during continued neuronal activity suggests that [K\textsuperscript{+}]\textsubscript{o}, is actively cleared from the extracellular space.

Clearing of excess [K\textsuperscript{+}]\textsubscript{o}, is believed to occur by diffusion, active uptake by neurons and glia, or passive uptake by glia (Newman 1995). There is evidence that the rate of potassium release during repetitive neuronal activity is faster than the rate at which it would diffuse away (Somjen 1979). Although neurons can take up potassium, uptake by astrocytes is believed to play a major role in regulation of [K\textsuperscript{+}]\textsubscript{o}. Glia are thought to be required for the normal fine tuning of [K\textsuperscript{+}]\textsubscript{o} and for the recovery of pathologically elevated [K\textsuperscript{+}]\textsubscript{o} (Francke et al. 1997; Largo et al. 1996). Glia have been shown to increase their internal potassium concentration when [K\textsuperscript{+}]\textsubscript{o} is increased and release it once the [K\textsuperscript{+}]\textsubscript{o} decreases (Ballanyi et al. 1987; Walz 1989). They are also believed to remove potassium by spatial buffering through the glial syncytium (Dietzel et al. 1989). According to the spatial buffering hypothesis (Gardner-Medwin 1983; Newman 1995; Orkand 1986), potassium released from active neurons enters glial cells, possibly through inwardly rectifying potassium channels. Potassium is then redistributed through the network of glial cells and leaves at sites of low [K\textsuperscript{+}]\textsubscript{o}. Spatial buffering can be directly demonstrated in the drone retina as a result of a fortunate spatial arrangement of neuronal and glial structures (Coles and Orkand 1983; Gardner-Medwin et al. 1981; Karwoski et al. 1980; Newman and Frambach 1984; Oakley et al. 1992). However, the exact role of glial spatial buffering in other parts of the brain and during times when the regulation systems are significantly stressed (i.e., during synchronous epileptiform activity) is not clear. It has been argued that spatial buffering has no role in situations of elevated [K\textsuperscript{+}]\textsubscript{o} (Barres 1991).

The purpose of the present experiments was to investigate regulation of [K\textsuperscript{+}]\textsubscript{o} during epileptiform activity in the dentate gyrus in vitro. Using potassium-sensitive microelectrodes, we examined the effects of blocking the inward rectifying potassium channel, blocking gap junctions, blocking chloride associated potassium uptake, blocking local neuronal activity, and blocking the sodium pump on the baseline [K\textsuperscript{+}]\textsubscript{o}, the ceiling level of [K\textsuperscript{+}]\textsubscript{o}, during epileptiform activity, and the rate of recovery of the [K\textsuperscript{+}]\textsubscript{o}, after termination of the epileptiform bursts. The results suggest that glial uptake mechanisms, including spatial buffering, are not the primary regulators of [K\textsuperscript{+}]\textsubscript{o}. In this model, but that active uptake via the sodium pump is of critical importance.

METHODS

All experiments were carried out in accordance with the NIH guide for the care and use of laboratory animals. Hippocampal slices were prepared by conventional methods from adult Sprague–Dawley rats (100–200 g, both sexes). After anesthetizing the rats with ketamine (25 mg/kg), xylazine (5 mg/kg), and acepromazine (0.8 mg/kg) i.p., the brains were removed. Transverse slices (400–500 μm) through the hippocampus were cut with a Vibratome (Technical Products International). Slices were placed in an interface-type chamber and con-
tuously perfused with artificial cerebrospinal fluid (ACSF) at 33°C under a stream of humidified 95% O₂-5% CO₂. Composition of the ACSF was (in mM) 127 NaCl, 2 KCl, 1.5 MgSO₄, 1.1 KH₂PO₄, 26 NaHCO₃, 2 CaCl₂, and 10 glucose. In experiments where barium was added to the perfusion solution, the MgSO₄ was replaced with MgCl₂. All solutions were bubbled constantly with 95% O₂-5% CO₂. Slices were allowed to equilibrate for 1 h before electrophysiological recording was begun.

Extracellular field potentials were recorded from the dentate gyrus of the hippocampus. Recording electrodes were made of microfilamentary thin-walled glass (A-M Systems, 0.9 mm OD, 1.2 mm ID) pulled on a micropipette puller (P-87, Sutter Inst.). Electrodes were filled with 2 M NaCl to give an impedance of 4–10 MΩ. Nonsynaptic epileptiform activity was induced in the dentate gyrus by changing to ACSF containing 0-added calcium and a total of 8 mM potassium (Pan and Stringer 1996; Schweitzer et al. 1992). This spontaneous epileptiform activity consists of recurrent bursts of 10–25 mV population spikes associated with a negative shift of the extracellular DC potential and a rise in the extracellular potassium concentration ([K⁺]ₒ). This triad of responses in the slice will be termed field bursts. The field bursts recur in a very stable pattern for 3–4 h. Between field bursts, there are nonsynchronous bursts of 2–5 action potentials in individual granule cells that occur at a regular interval (between 5–7 Hz) (Pan and Stringer 1996) and will contribute to the baseline level of [K⁺], that is recorded between field bursts. During the field bursts there is continuous action potential firing at a rate of ~25 Hz, suggesting a maintained neuronal outflow of potassium. The termination of the field bursts is quite synchronous, i.e., the entire dentate gyrus stops firing at the same time. After burst termination the granule cells are silent for 2–4 s before the baseline activity starts up again.

DC-coupled recording of field potentials and [K⁺]ₒ were carried out with double-barreled ion sensitive electrodes (Stringer and Lothman 1989). One barrel was silanized with 15% tri-N-butylchlorosilane (Alfrebro, Monroe, OH) in chloroform and the tip was filled with the potassium selective resin (Fluka Cocktail “B” or Corning 477317). The electrode was then backfilled with 1 M potassium acetate. The reference barrel was filled with 2 M NaCl. The reference and potassium signals were amplified and displayed on a chart recorder. Both ion exchange resins were used because they have different sensitivities. The Corning exchange is more sensitive to NH₄⁺, which is used in some of the experiments, and acetylcholine. The Fluka exchange is more selective but gives higher resistance electrodes. Both types of electrodes were used in every experiment in this study and the results were comparable. Every electrode was calibrated before the experiment in a series of standard solutions in ACSF (2, 3, 5, 10, and 20 mM potassium) at 33°C and the individual calibrations were used to determine the [K⁺]ₒ for each experiment. The electrode tip diameter was 2–3 μm and electrodes had an impedance of around 10⁶ Ω. Electrodes were not used for this study unless they had ≥50 mV change between the 2 and 20 mM potassium standards and had a time constant of ≤50 ms in response to a rapid step increase in [K⁺]ₒ. For the experiments that used cesium in the perfusing solution, the potassium-sensitive electrodes for these experiments were also calibrated in standard solutions containing ACSF with 3 mM CsCl and the various potassium concentrations. Electrodes made with both ion exchange resins were sensitive to cesium, resulting in a higher electrical potential for each [K⁺]₉. However, the sensitivity of the electrodes to changes in [K⁺]ₒ was not altered, i.e., the change in electrical potential between standards was the same in the presence and absence of cesium. During each experiment, the electrode was also calibrated by moving from the slice to perfusion solution and comparing the bath and tissue potentials. Recordings in the slices were made 50–100 μm below the slice surface. The maximal [K⁺]ₒ reached during field bursts, which are 20–30 s in duration, was taken as the ceiling level. The [K⁺]ₒ level exactly half way between two field bursts (≥20 s apart) was taken as the baseline level. The half-time of [K⁺]ₒ recovery was taken as the time for the [K⁺]ₒ to fall to one half the original concentration. The half-time of recovery of the [K⁺]ₒ was measured immediately after burst termination to try to measure it during the period in which the neurons are silent. After the baseline neuronal activity recurs, it may slow the rate of recovery by adding to the extracellular potassium.

All compounds were purchased from Sigma (St. Louis, MO). In all experiments the recurrent nonsynaptic field bursts were induced by perfusing with 0-added calcium and 8 mM potassium medium. Additional drugs were then added to this solution. For some experiments, drugs were applied locally by leakage from a glass pipette (3–5 μm tip diam) lowered into the slice.

RESULTS

Effect of local application of tetrodotoxin

To determine whether [K⁺]ₒ is moved from areas of high concentration to areas of low concentration during the field bursts, the spontaneous regular field bursts were initiated by perfusing with 8 mM potassium and 0-added calcium and then a laminar analysis of the peak ceiling level reached during the bursts was carried out to map the areas of high and low [K⁺]ₒ. Preliminary experiments demonstrated that placement of the potassium-sensitive electrode in any part of the dorsal leaf of the dentate gyrus gave the same measurements for the changes of the [K⁺]ₒ. Also, there was remarkable agreement across slices for the half-time of recovery of [K⁺]ₒ, in spite of differences in burst duration between slices. Analysis showed that the ceiling level of [K⁺]ₒ was maximal in the granule cell layer and declined toward the hilus and molecular layer (n = 5, Fig. 1). The baseline level of [K⁺]ₒ between field bursts was the same as the perfusing solution in all areas and the half-time of recovery of [K⁺]ₒ from the ceiling level was the same in all areas (mean 3.7 s, range 3.6–3.8 s). The [K⁺]ₒ increases in the hilar and molecular regions could be caused by diffusion (or spatial buffering) from the area of highest concentration (cell layer) or could be caused by local release of potassium from activity in interneurons, axons, and/or dendrites. Estimates of the space constant of the astrocytic syncytium suggest that the distance over which the potassium spatial buffering process is effective is limited to hundreds of microns (Newman 1995), which is about the distance to the furthest extent of the molecular and hilar regions. However, the prompt rise of the [K⁺]ₒ, in each area (without delay) suggests that local release accounts for the rise in [K⁺]ₒ.

To directly test whether potassium is released and taken up locally, we applied tetrodotoxin (TTX) locally and measured local changes in [K⁺]ₒ. TTX is a sodium channel blocker that will block action potentials and local neuronal activity. A pipette was filled with 1 mM TTX in 0-added calcium and 8 mM potassium ACSF, the tip broken off so that the TTX would leak out, and placed adjacent to the potassium-sensitive electrode (either the hilar area, n = 3 or the molecular layer, n = 3). A reference electrode was maintained in the granule cell layer to be sure that the TTX was not perfusing to the cell layer. As shown in Fig. 2, local application of TTX blocked most of the increase in [K⁺]ₒ and reduced the amplitude of the epileptiform activity, whereas there was no change in the amplitude or DC shift of the recording in the granule cell layer. The remaining [K⁺]ₒ increase may be caused by passive diffusion or glial buffering from neighboring areas, but the ma-
jority of the $[K^+]_{o}$ increase has been blocked. Control experiments in which the TTX was applied to the cell body layer showed that the amplitude of the DC shift decreased in conjunction with the loss of the rise in $[K^+]_{o}$. Thus monitoring of the extracellular DC potential in the cell body layer is a reasonable control that the $[K^+]_{o}$ levels in the cell layer are not being altered by the TTX application in the hilar or dendritic regions. For additional control experiments, the pipette was filled with ACSF with 0-added calcium and 8 mM potassium and the tip was broken back. Placement of this pipette in the slice (either cell layer, $n = 3$, dendritic region, $n = 3$, and hilus, $n = 3$) had no effect on the $[K^+]_{o}$ measurements or the amplitude of the field burst.

**Effect of blockade of the inward rectifying potassium channel**

We next tested the effects of blockers of the inward rectifying potassium channels, cesium and barium (Ransom and Sontheimer 1995), on extracellular potassium regulation during and after epileptiform activity in the dentate gyrus. Initial concentrations for cesium and barium were based on the $IC_{50}$'s reported by Ransom and Sontheimer (1995). The effect of barium (a voltage-independent blocker) on the regulation of $[K^+]_{o}$ was determined (Fig. 3A). Barium (0.2–0.3 mM) did not alter the ceiling level of $[K^+]_{o}$ during the field bursts nor the half-time of recovery after the field bursts ($n = 5$). The half-time of recovery after 0.2 mM barium was $3.91 \pm 0.47$ (SE) s compared with $3.75 \pm 0.43$ before barium ($n = 5$ for both). Increasing the barium concentration to 0.5 mM increased excitability of the tissue, as indicated by a reduction in the time between field bursts, and induced spreading depression (data not shown, $n = 5$) making measurements of half-time of recovery and baseline $[K^+]_{o}$ impossible with the higher concentrations.

To confirm the results with barium, the effects of cesium (a voltage-dependent blocker) were determined. Addition of cesium (1–3 mM) to 8 mM potassium ACSF caused the field bursts to occur too close together for accurate measurements of baseline and half-time of recovery ($n = 8$). Therefore to test the effect of cesium on $[K^+]_{o}$ regulation, the $[K^+]_{o}$ in the ACSF was lowered to 6 mM after the induction of the field bursts. After 30 min in 6 mM $[K^+]_{o}$, cesium (2 mM) was added to the perfusing solution and the field bursts reappeared within minutes. Cesium did not significantly change the baseline $[K^+]_{o}$ level, the ceiling $[K^+]_{o}$ level nor did it slow the half-time of recovery of $[K^+]_{o}$ after the field bursts ($3.66 \pm 0.31$ s compared with $3.69 \pm 0.54$ s before cesium, $n = 12$, Fig. 3B).

**Effect of gap junction blockers**

If gap junctions between glial cells are critical for the spatial buffering of the $[K^+]_{o}$, then reduction of gap junction conductance should alter the regulation of the $[K^+]_{o}$ during and after the field bursts. Preliminary experiments with the gap junction blocker octanol (de Curtis et al. 1998; Finkbeiner 1992) showed that 0.5 mM (or higher) completely and quickly (< 10 min) blocked the field bursts. However, it appeared that during perfusion with 0.5 mM octanol, that the half-time of recovery for the $[K^+]_{o}$ was not changing with the burst duration. Lower doses (0.05 to 0.2 mM) had no effect on the baseline level of $[K^+]_{o}$ or the half-time of recovery of $[K^+]_{o}$ after the burst. The half-time of recovery after 0.1 mM octanol was $3.65 \pm 0.4$ s compared with $3.65 \pm 0.29$ s before octanol ($n = 5$). The duration of the field bursts was shortened by octanol (0.05–0.2 mM) and as the burst duration shortened, the ceiling $[K^+]_{o}$ during the bursts did decrease ($n = 11$, Fig. 4).

The results with octanol were confirmed with by altering gap junction with ammonium chloride (Perez-Velazquez et al. 1994; Tolkovsky and Richards 1987). Administration of ammonium chloride causes a transient intracellular alkalinization and therefore an increase in conductance through gap junc-
tions. Washout of the ammonium chloride causes a transient intracellular acidification and decrease in gap junction conductance. At 10 mM, continuous epileptiform activity was initiated and it was impossible to determine potassium regulation, whereas, at 1 mM ammonium chloride had no effect. Perfusion with 3 mM caused no change in the ceiling level of \([K^+]_o\) \((n = 6)\), but it was not possible to measure the half-time of recovery because of the presence of an afterdischarge. During washout of the ammonium chloride there was a decrease in the ceiling level of \([K^+]_o\). There was no change (compared with normal pH) in the baseline level of \([K^+]_o\) or in the half-time of recovery of the \([K^+]_o\) \((3.75 \text{ s}, \ n = 2)\). The effects of the washout of ammonium chloride, which should decrease gap junction conductance, are entirely consistent with the effects of octanol, a gap junction blocker.

**Effect of altering Cl⁻ dependent potassium uptake**

An alternative mechanism for \([K^+]_o\) homeostasis proposes that glial cells act to temporarily sequester potassium by the uptake of KCl. The Cl⁻ ions passively follow the K⁺ ions into the cells, resulting in the net accumulation of KCl. We tested this idea by using furosemide (K⁺/Cl⁻ cotransporter and Na⁺/K⁺/2Cl⁻ cotransporter inhibitor) to block Cl⁻ dependent K⁺ uptake and by lowering the extracellular Cl⁻ concentration (Geck and Pfeiffer 1985; Hochman et al. 1995, 1999). Concentrations of furosemide and low chloride were obtained from Hochman et al. (1995, 1999).

Perfusion with ACSF containing furosemide (2–10 mM) decreased the time interval between bursts and shortened the bursts in the first 10–40 min of the perfusion. Prolonged perfusion with furosemide caused complete block of the field bursts with all doses tested. Lower doses of furosemide required longer perfusion for the blockade compared with higher doses. Furosemide at 2 mM \((n = 3)\) blocked field bursts after 3 h, whereas 5 mM required only 1–2 h \((n = 6)\). Furosemide did not alter the baseline level of \([K^+]_o\) and did not slow the rate of potassium clearance (Fig. 5). After 30 min of perfusion with 5 mM furosemide the half-time of recovery was 3.74 ±
0.37 s compared with 3.68 ± 0.4 s before furosemide (n = 6). Early in the perfusion there was an increase in the ceiling level of [K+]o during the field bursts. Later in the perfusion, as the field bursts shortened significantly, there was a decrease in the ceiling level of [K+]o.

Lowering the extracellular chloride concentration from 138 mM to 20 mM had similar effects as furosemide (n = 4, Fig. 6). To maintain the osmolality of the perfusing solution, sodium gluconate was substituted for removed NaCl in the perfusate (Avoli et al. 1990). During perfusion with low chloride, there was no change in the baseline level of [K+]o nor in the half-time of recovery of the [K+]o after the bursts. The half-time of recovery was 3.82 ± 0.4 s after perfusion with low chloride compared with 3.80 ± 0.33 s before switching to low chloride (n = 4). As with furosemide, there was an increase in the ceiling level during the field bursts.

**Effect of inhibition of the sodium pump**

Both neurons and glia have an enzyme (a Na+–K+–ATPase) that actively pumps sodium out and potassium back into the cell. To determine whether this enzyme plays a role in the regulation of the [K+]o during and after seizures we tested the effect of ouabain, a specific Na+–K+–ATPase inhibitor (Lingel 1992). Concentrations ≥20 µM were tried. Perfusion with concentrations of ouabain ≥5 µM caused an increase in [K+]o within minutes that never recovered (n = 12). No neuronal responses could be evoked and there was no recovery of the [K+]o toward baseline within 30 min of recording. Addition of 5 µM ouabain (n = 11), caused an immediate decrease in the interval between field bursts followed by spreading depression in all slices (Fig. 7). At this concentration of ouabain, ~50% of the time the slice recovered from the spreading depression.
within 5 min. Ouabain (5 μM) increased the baseline level of \([K^+]_o\) (before; 8.05 ± 0.04 mM, ouabain; 9.87 ± 0.12 mM, \(P < 0.001, n = 6, \text{paired } t\)-test) and ceiling \([K^+]_o\) (before; 15.02 ± 0.23 mM, ouabain; 16.35 ± 0.48 mM, \(P < 0.05\)). Ouabain also decreased the half-time of \([K^+]_o\) recovery after the field bursts (before; 3.8 ± 0.4 s, ouabain; 7.3 ± 0.5 s, \(P < 0.001\)).

To try to avoid the initiation of spreading depression, ouabain was locally applied into the hilus \((n = 10)\) or the molecular layer \((n = 11)\). A pipette was filled with 5 mM ouabain in 0-calcium and 8 mM potassium ACSF and the tip cut back so that the pipette leaked ouabain. As with the TTX, a reference electrode was placed in the granule cell layer to detect diffusion of the ouabain. If the pipette was filled with higher concentrations of ouabain \((>5 \text{ mM})\), within seconds of placement of the pipette in the tissue spreading depression was triggered and the duration of the spreading depression was prolonged, sometimes with no recovery. Local application of ouabain \((5 \text{ mM})\) caused an increase in both baseline and ceiling \([K^+]_o\) and a decrease in the half-time of recovery without significantly altering the epileptiform activity in the granule cell layer (Fig. 8). The effects of ouabain were partially reversible when the ouabain pipette was removed. As with the TTX experiments, placement of a pipette containing ACSF had no effect on the \([K^+]_o\) measurements.

**DISCUSSION**

Clearance of excess potassium during intense neuronal activity and after its termination has been postulated to be caused by diffusion, uptake by glia, or uptake by neurons. We have tested the role of astrocytes in the regulation of the baseline \([K^+]_o\), ceiling level of \([K^+]_o\) during synchronous epileptiform activity, and the rate of recovery of the \([K^+]_o\) from elevated levels. For this we have used a model of regularly recurring spontaneous bursts of population spikes recorded in the dentate gyrus of hippocampal slices. Overall, the results suggest that glia play a relatively minor role in regulation of the baseline levels of \([K^+]_o\) and in the recovery of elevated \([K^+]_o\) levels. Chloride-dependent uptake of potassium may have a role in determining the level of \([K^+]_o\) during the field bursts, but a major regulator of the ceiling level and the recovery in this model appears to be uptake via the \(
\text{Na}^+-\text{K}^+-\text{ATPase}\). Because initiation of the recurrent epileptiform activity in this model requires perfusion with elevated potassium \((8 \text{ mM})\), it may be that the regulatory mechanisms are not the same as in the intact brain. Experiments in the dentate gyrus in situ would be helpful.

Of the three measurements of \([K^+]_o\) regulation, interpretation of the changes in half-time of recovery are the simplest. The half time of recovery of the \([K^+]_o\) was taken as a measure of the ability of the brain tissue to reduce elevated levels of \([K^+]_o\). During this time the neurons have stopped firing and there is little potassium movement out of cells. Because the \([K^+]_o\) is still elevated above the concentration in the perfusing solution, mechanisms should be active that reduce \([K^+]_o\). In the present experiments, only inhibition of the sodium pump significantly altered the half-time of recovery of \([K^+]_o\), slowing it at low doses of ouabain, partial inhibition of the pump (Lingel 1992), and completely blocking recovery at higher doses. It would appear that passive glial uptake mechanisms,
including spatial buffering, have no role in this phase of potassium regulation in this model. A number of other studies have reported a slowing of the half-time of recovery of \([K^+]_o\) from elevated levels and an increase in the baseline (unstimulated levels) of \([K^+]_o\) after inhibition of the sodium pump (Arens et al. 1992; Haglund and Schwartzkroin 1990; Krnjević and Morris 1975; McCarren and Alger 1987). In fact, one study used the rate of clearance of \([K^+]_o\) as an indicator of the pump activity (McCarren and Alger 1987). Additionally, the rapid recovery of \([K^+]_o\) has been cited as evidence for neuronal uptake (Cordingley and Somjen 1978). As further evidence that the sodium pump may be a major factor in the regulation of \([K^+]_o\), simultaneous recordings of \([K^+]_o\) and extracellular sodium have shown that the time course of recovery of both are about equal (Arens et al. 1992; Dietzel 1982). This suggests that movement of sodium back out of cells is balanced by movement of potassium into cells, possibly via the sodium pump. In contrast, Gardner-Medwin (1983) suggests that active uptake into neurons will occur slowly, with a time constant in the tens of seconds, much longer than the rate of recovery seen in the present experiments.

Changes in the ceiling level of \([K^+]_o\) are more difficult to interpret as a result of the multiple processes ongoing during this time period. When the \([K^+]_o\) is at the ceiling level (during the field bursts), the neurons continue to fire at relatively high frequency and release potassium into the extracellular space. Therefore to maintain the ceiling level, there must be removal of potassium from the extracellular space that matches the neuronal output. Changes in the ceiling level for \([K^+]_o\) may be caused by changes in the size of the extracellular space, changes in the amount of potassium released by the neurons, or changes in one or more uptake mechanisms. In these experiments the ceiling level was reduced by local TTX application and perfusion with gap junction inhibitors; both results are consistent with a decrease in neuronal output. The experiments with gap junction blockers suggest that gap junctions between glia are not of primary importance in determining the ceiling level of \([K^+]_o\), because blocking glial gap junctions would be predicted to reduce spatial buffering and increase the ceiling, the exact opposite to what was measured in these experiments. The ceiling level was increased by inhibition of the Na\(^+/\)K\(^+\)-ATPase and inhibition of Cl\(^-\) dependent uptake. This increase may be a result of a number of factors including a change in extracellular space. Previously, furosemide has been postulated to block seizures by inhibiting cell swelling, resulting in a larger extracellular space (Hochman et al. 1995). The ceiling level of \([K^+]_o\) was not changed by blocking the inward rectifier suggesting that glial uptake of potassium is not critical for determination of the ceiling level.

Baseline levels of \([K^+]_o\) in the slice are the most difficult to
measure and interpret. Slower processes that may regulate \([K^+]_o\) over the time span of minutes will not be detected in this in vitro model because of the perfusion of ACSF. The perfusing solution takes away excess potassium and will add potassium if the level in the slice falls below the ACSF level. To control for drift of the electrical potential through the potassium-sensitive electrode, all baseline measurements in the slice were compared with the perfusing solution. However, there is still the problem of when to measure. Most often (see Fig. 5), there is an overshoot of the \([K^+]_o\) below the level of the perfusing solution after each field burst and then a gradual increase. The closer together the field bursts, the harder it is to determine any baseline level. Therefore small or subtle changes in the baseline level of \([K^+]_o\), are not detectable with this system. However, inhibition of the sodium pump increased the baseline nearly 2 mM. This suggests that the baseline \([K^+]_o\), during the recurrent field bursts in this model is regulated, in part, by the active uptake of potassium through the sodium pump. These experiments do not rule out that other mechanisms may also contribute.

It is not clear from these experiments alone, whether it is the glia (presumably astrocytes), the neurons, or both that are most important in the regulation of \([K^+]_o\). The chloride-dependent uptake is thought to occur in both neurons and glia. Na\(^+\)/K\(^+\)-ATPase activity is found in high concentration both in neurons, where it maintains the sodium/potassium gradient essential for nerve impulse generation, and in glial cells where it is believed to be involved in buffering \([K^+]_o\) (Lingel 1992; Walz 1989). Increases in \([K^+]_o\) would activate both glia and neuronal enzymes (Lingel 1992). However, the sodium influx into neurons during neuronal activity, which would not occur in glia, is a potent activator of the neuronal sodium pump. In addition, if most of the potassium released by neurons was siphoned away, then it is hard to imagine that neurons could sustain such prolonged discharges (tens of seconds) without a significant fall in intracellular potassium. Finally, we have recently shown that there is little change in the regulation of \([K^+]_o\) in vivo in the absence of functional astrocytes (Xiong and Stringer 1999).

Although not direct evidence, altogether this information suggests that neuronal uptake of potassium is critical to the regulation of \([K^+]_o\) in this model.

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Address for reprint requests: J. L. Stringer, Dept. of Pharmacology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

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