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Characteristics of activity-dependent potassium accumulation in mammalian peripheral nerve in vitro

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Ion-sensitive microelectrodes were used to study the behavior of extracellular ions in rat sciatic nerve during and following activity. Nerve stimulation produced increases in $[K^+]_o$ that were dependent upon the frequency and duration of stimulation; no change in extracellular pH occurred with stimulation. Increases in $[K^+]_o$ depended on axonal discharge since they were blocked by inhibiting sodium channels with tetrodotoxin. At 22 °C, stimulation could induce increases in $[K^+]_o$ of several mM; at 36 °C, stimulation rarely produced increases in $[K^+]_o$ greater than 1 mM. Stimulated increases in $[K^+]_o$ dissipated very slowly (i.e. $t_{1/2}$ = 50–100 s) and the rate of dissipation was not significantly affected by anoxia, changes in temperature, changes in extracellular pH, or the application of a blocker of Na^+, K^+-ATPase (ouabain) or a K^+ channel blocker (Ba^{2+}). In comparison to the central nervous system, neural activity in rat sciatic nerve produced smaller increases in $[K^+]_o$ and these increases dissipated much more slowly. The primary mechanism of K^+ dissipation appeared to be diffusion, probably facilitated by the larger extracellular space in peripheral nerve compared to the central nervous system, but impeded by diffusion barriers imposed by the blood–nerve barrier.

INTRODUCTION

Action potentials in axons can result in accumulation of K^+ in the extracellular fluid surrounding the active membrane. This was first anticipated on the basis of the progressive decline in the positive after-potentials seen with repetitive action potentials in the squid giant axon[16]; it was reasoned that K^+ leaving the axon during the repolarization phase of each action potential encountered a diffusion barrier in the form of a tight glial ensheathment and that this led to a transient build-up of extracellular K^+ concentration ($[K^+]_o$; see ref. 3). The importance of activity-dependent extracellular K^+ accumulation is that such accumulations may give rise to physiologically important interactions affecting neuronal excitability and synaptic efficacy[1,14,44,49]. In the mammalian central nervous system (CNS), neural activity results in K^+ accumulation because of a very restrictive extracellular space (e.g. ref. 9); the time course and magnitude of K^+ accumulation within the CNS, as well as the mechanisms that underlie dissipation, have been intensively investigated[31,45]. The rate of dissipation of activity-dependent $[K^+]_o$ increases in the CNS is too rapid to be explained by diffusion alone and has been shown to be an energy-dependent process[9,31,45].

In mammalian peripheral nerve, the characteristics of K^+ accumulation with axonal activity have not been well studied. The extracellular space surrounding axons in peripheral nerves is less restrictive than within the brain and this may strongly affect the extent of K^+ build-up[36]. Nevertheless, small K^+ accumulations occur in association with tetanic stimulation of frog sciatic nerve[28] and rat spinal root axons[6], and large K^+ accumulations follow activity in the completely unmyelinated vagus nerve[15]. Convincing data show that changes in extracellular K^+ can alter peripheral nerve function[28]. We studied the features of activity-dependent $[K^+]_o$ accumulation and dissipation in rat sciatic nerve and found that it differed in important regards from K^+ accumulation in rat CNS (including white matter tracts) and vagus nerve; in sciatic nerve, K^+ accumulated to a lesser extent and there was no evidence of energy-dependent removal mechanisms.

MATERIALS AND METHODS

Sciatic nerves were obtained primarily from adult Long–Evans rats; a small number of experiments were done on 6-day-old rats. The nerve was dissected free and placed in a standard brain slice recording chamber maintained at 22 or 36 °C. The chamber was continuously perfused with a standard solution containing in mM: NaCl 130, KCl 3, NaH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, dextrose 10,

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HEPES 10. The solution was gased with O_2 and pH was adjusted to 7.4 with NaOH. To test the effects of HCO_3^- concentration, the following solution was used (in mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, dextrose 10 and pH was adjusted to 7.4 by continuous aeration with 5% CO_2 and 95% O_2. To lower pH in the solution, HCl was added. In the solution containing 5 mM Ba^{2+}, it replaced the following salts: 2 mM $MgSO_4$, 1.25 mM NaH_2PO_4 and 1.75 mM NaCl. For some experiments TTX (2 μM) and ouabain (0.1 mM) (Sigma, Taufkirchen, F.R.G.) were added directly to the standard solution.

Double-barrelled K^+-sensitive microelectrodes were prepared according to a method described by Munoz and Coles[29]. Pipettes were pulled from theta glass and broken to a tip diameter of about 5 μm. Although tip diameters of this size tend to underestimate the size of transient increase in $[K^+]_o$, the magnitude of this effect is not large[39]. The reference barrel of the electrode was filled with 0.15 M NaCl, while the sensitive barrel was filled with K^+ exchanger obtained either from Fluka (Neu-Ulm, F.R.G.) or prepared from 3% by weight K^+-tetra-*p*-chlorophenylborate in 2.6-dimethylnitrobenzene (Corning-type), and backfilled with 0.5 mM KCl. Electrodes were calibrated in solutions containing between 3 and 10 mM KCl. Double-barrelled pH-sensitive microelectrodes were made in a similar fashion using the Fluka proton cocktail[2].

The sciatic nerve trunk was stimulated with a suction electrode connected to a Grass SD9 stimulator. The voltage signals from the ion-selective electrodes were amplified and displayed by conventional equipment. In addition, data were digitized and stored on an AT-compatible computer. With the aid of programs developed at the University of Heidelberg, data could be displayed, processed and printed.

RESULTS

Characteristics of activity-dependent K^+ accumulation

To measure $[K^+]_o$ in the sciatic nerve, K^+-selective microelectrodes were inserted into about the middle of the nerve. Experiments were done at room temperature (i.e. 22 °C) unless otherwise specified. The position of the microelectrode was adjusted so that it recorded the largest possible compound action potential (CAP) in response to a single supramaximal stimulus. Repetitive stimulation of the sciatic nerve by trains of stimuli resulted in an increase in $[K^+]_o$ (Figs. 1 and 2) and the magnitude of K^+ accumulation depended upon train duration (Fig. 1) and train frequency (Fig. 2). The maximum stimulated increase in $[K^+]_o$ seen under the conditions of these experiments was approximately 5 mM to an absolute $[K^+]_o$ of 8 mM (Fig. 2). With our standard stimulus parameters (i.e. 120 Hz, 10 V, 150 ms stimulus duration, and 120 s of stimulation), $[K^+]_o$ increased by an average of 1.9 ± 1.0 mM (n = 6). Several 6-day-old sciatic nerves were studied in a similar fashion and never showed activity-dependent accumulation of $[K^+]_o$ to a level greater than 1 mM (not illustrated).

The rate of K^+ dissipation following activity-dependent increases in $[K^+]_o$ was very slow in the sciatic nerve when recordings were done at 22 °C (Figs. 1–3). In marked contrast to central white matter tracts such as the rat optic nerve, no clear undershoots in $[K^+]_o$ followed intense periods of stimulation[9,15]. The kinetics of $[K^+]_o$ decay after an evoked increase in the sciatic nerve are shown in Figs. 3–5. The half-time of dissipation was often greater than 100 s and was not significantly influenced by the magnitude of the preceding K^+ accumulation (Fig. 3; cf. ref. 25).

The temperature coefficient (or Q_{10}) of active metabolic processes is high and high Q_{10}s have been found for K^+ removal in the rat optic nerve, guinea pig hippocampal slice (Ransom, unpublished), cat cortex in vivo[26] and cat spinal cord[46], implying that K^+ removal is an

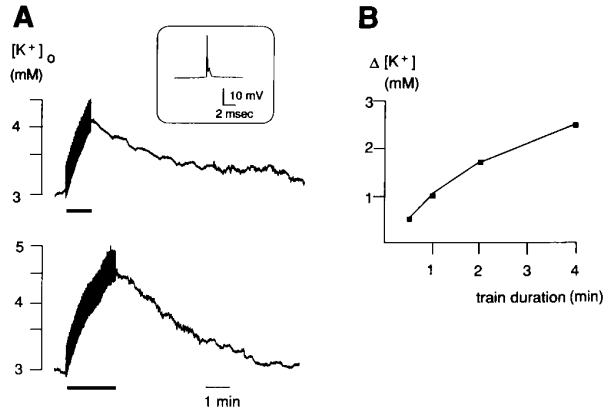

Fig. 1. Effect of stimulus train duration on changes in extracellular K^+ concentration ($[K^+]_o$) in rat sciatic nerve. A: trains of stimuli were applied (indicated by bars) with a frequency of 120 Hz. In the upper trace the train had a 60-s duration, in the lower trace a 120-s duration. $[K^+]_o$ was measured by a microelectrode inserted into the middle of the nerve. The position of the electrode was adjusted to optimize the recording of the compound action potential (inset on top). B: from recordings as shown in A, maximum $[K^+]_o$ increase ($\Delta[K^+]$) was plotted as a function of train duration for a single representative nerve.

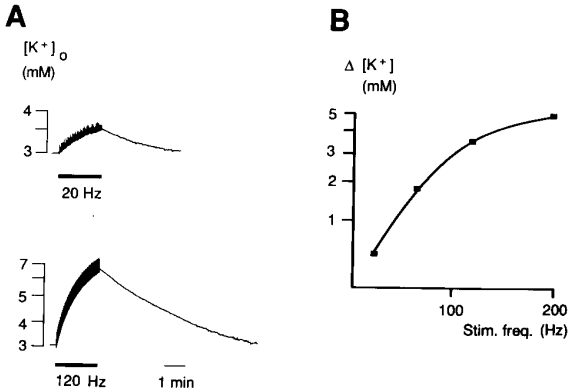

Fig. 2. Effect of frequency of train stimuli on changes in $[K^+]_o$. A: $[K^+]_o$ was recorded as described in the legend to Fig. 1. The frequency of stimulation was varied as indicated. Train duration was kept constant at 120 s. B: from recordings as shown in A, maximum $[K^+]_o$ increase ($\Delta[K^+]$) was plotted as a function of stimulus frequency for a single representative nerve.

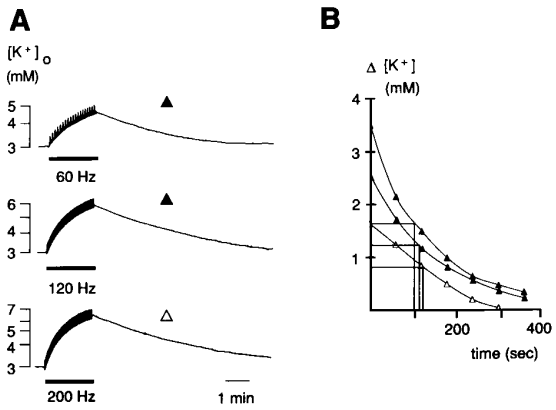

Fig. 3. The effect of frequency of train stimuli on the dissipation of $[K^+]_o$. A: stimulus-induced changes in $[K^+]_o$ were recorded as described in the legend to Fig. 1. Train duration was kept constant at 120 s and the frequency was varied as indicated. B: for the period following stimulation, changes in $[K^+]_o$ were determined relative to the baseline prior to stimulation ($\Delta[K^+]$) from recordings as shown in A. $\Delta[K^+]$ was plotted as a function of time after stimulus termination. The time at which $\Delta[K^+]$ decreased to half of its peak value ($t_{1/2}$) is indicated by the lines. Note that ($t_{1/2}$) is independent of stimulus frequency.

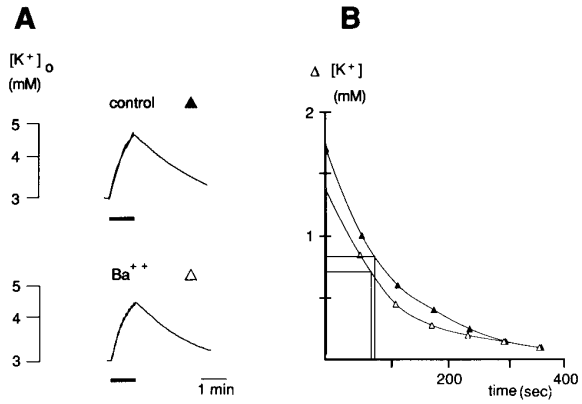

Fig. 5. Effect of Ba^{2+} on stimulus-induced changes in $[K^+]_o$. A: stimulus-induced changes in $[K^+]_o$ were recorded as described in the legend to Fig. 1. Recordings were made in standard solution (upper trace) and in a Ba^{2+}-containing solution (lower trace). B: the dissipation of the evoked $[K^+]_o$ changes were plotted as a function of time as described in the legend to Fig. 3B. The filled triangles show the time course in standard solution, the open triangles in Ba^{2+}-containing solution.

active process in these preparations (cf. ref. 7). Increasing the temperature of rat sciatic nerves from 22 to 36 °C, however, did not significantly change the rate of K^+ removal after stimulation-dependent build-up (Fig. 4). Under conditions of constant stimulation intensity, 5 nerves were studied at 22 °C and then at 36 °C; the average half-time of dissipation ($t_{1/2}$) at 36 °C was 70 ± 20 (S.D.) s, and the average $t_{1/2}$ at 22 °C was 58 ± 25 (S.D.) s. At 37 °C the average $t_{1/2}$ of K^+ dissipation in adult rat optic nerves was 4.8 s[9]; this is markedly different from the average half-time found in the sciatic nerve at 36 °C, i.e. 70 ± 20 s.

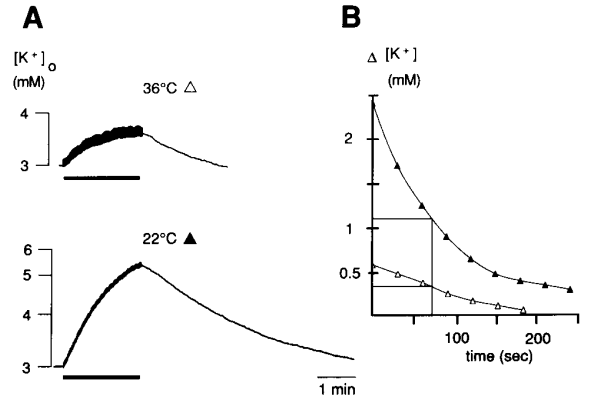

Fig. 4. Effect of temperature on stimulus-induced changes in $[K^+]_o$. A: stimulus-induced (indicated by bars) changes in $[K^+]_o$ were recorded as described in the legend to Fig. 1. The stimulation parameters were: 120 Hz, 10 V, 150 ms pulse duration, train duration 2 min. The temperature of the nerve was varied as indicated. B: the dissipation of the evoked $[K^+]_o$ changes were plotted as a function of time as described in the legend to Fig. 3B. The open triangles show the time course at 36 °C, the filled triangles at 22 °C.

It was noted that the magnitude of stimulation-induced K^+ accumulation was consistently greater at lower temperatures (e.g. Fig. 4). The average value of maximum K^+ accumulation at 22 °C was 1.6 ± 0.9 mM compared to 0.48 ± 0.32 mM at 36 °C ($n = 5$; $P > 0.02$).

Effects of barium and TTX on activity-dependent increase in $[K^+]_o$

To verify that the stimulus-induced increases in $[K^+]_o$ were caused by action potentials in fibers of the sciatic nerve, we blocked action potential generation using the sodium channel blocker tetrodotoxin (TTX; 2 μM). As expected, both the CAP and the stimulus-induced increase in $[K^+]_o$ were blocked after incubating the nerve with TTX for 40 min.

In the mammalian, CNS the K^+ channel blocker Ba^{2+} causes an increase in activity-dependent K^+ accumulation (refs. 5,33; Carlini and Ransom, unpublished data). When 5 mM Ba^{2+} was applied to the sciatic nerve in 4 separate experiments, there was often a slight decrease in baseline $[K^+]_o$ (cf. ref. 5) and the amount of K^+ accumulation seen with the standard stimulus train was unchanged or slightly decreased (Fig. 5); the time course of K^+ dissipation following activity-dependent increase in the presence of Ba^{2+} was not significantly changed (Fig. 5).

pH_o and activity-dependent increases in $[K^+]_o$

Recent evidence has established that voltage-depen-

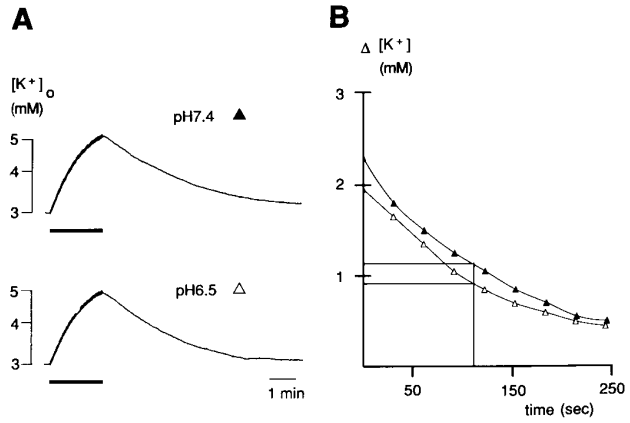

Fig. 6. Effect of altered extracellular pH on stimulus-induced changes in $[K^+]_o$. A: stimulus-induced changes in $[K^+]_o$ were recorded as described in the legend to Fig. 4. Recordings were made in standard solution (pH 7.4 upper trace) and in a solution buffered to pH 6.5 (lower trace). B: the dissipation of the evoked $[K^+]_o$ changes were plotted as a function of time as described in the legend to Fig. 3B. The filled triangles show the time course in pH 7.4 (standard solution), the open triangles in pH 6.5.

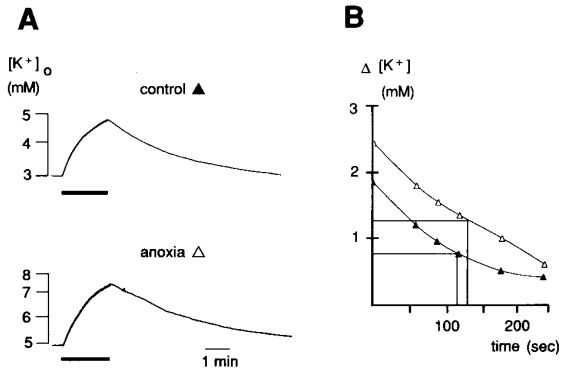

Fig. 7. Effect of anoxia on stimulus-induced changes in $[K^+]_o$. A: stimulus-induced changes in $[K^+]_o$ were recorded as described in the legend to Fig. 1. Recordings were made in standard solution (upper trace) and in a solution equilibrated with 95% N_2 and 5% CO_2 to obtain anoxic conditions ('anoxia'). B: the dissipation of the evoked $[K^+]_o$ changes were plotted as a function of time as described in the legend to Fig. 3B. The filled triangles show the time course of recordings in standard solution, the open triangles under anoxic conditions.

dent ion channels[18,19] and neuronal excitability[4,6] may be influenced by extracellular pH (pH_o). We therefore sought to determine in the sciatic nerve if there were activity-dependent changes in pH_o, as have been reported in rat vagus nerve[12], and if activity-dependent $[K^+]_o$ accumulation was influenced by changes in pH_o.

Microelectrodes sensitive to pH were inserted into the nerve to monitor pH_o during neural activity. Under stimulation conditions that elicited large increases in $[K^+]_o$, no changes in pH_o were noted (not illustrated), as was the case in rat spinal root[6]. This is in contrast to rat optic nerve, a CNS white matter tract, where large acid shifts in pH_o (up to 0.3 pH unit) are seen with similar stimulus trains (Carlini and Ransom, unpublished data), and rat vagus nerve where small acid shifts are recorded[12].

The effect of switching from the normal bath pH of 7.4 to a more acid pH of 6.5 (achieved by the addition of strong acid) on activity-dependent K^+ accumulation is shown in Fig. 6. Acidification had no effect on the magnitude of K^+ accumulation (average increase = 1.75 mM at pH 7.4 and 1.6 mM at pH 6.5, $n = 4$) or the rate of K^+ decay (average $t_{1/2}$ of K^+ dissipation = 76 s ± 19 s at pH 7.4 and average $t_{1/2}$ = 89 ± 9 s at pH 6.5, $n = 4$).

Decay of stimulus-induced increases in $[K^+]_o$: effects of ouabain and anoxia

It is well established that decay of activity-dependent increases in $[K^+]_o$ within the mammalian CNS uses energy-dependent processes[9,10,17,26,45]. In rat sciatic nerve, the slow time course of extracellular K^+ dissipation after stimulus-evoked increases, and the lack of post-stimulus undershoots in $[K^+]_o$ suggested that diffusion may be the predominant mechanism by which excess extracellular K^+ was removed (cf. ref. 7). To determine if energy-dependent processes affected K^+ dissipation in the sciatic nerve, we studied the effects of ouabain and anoxia.

Cardiac glycosides such as ouabain slow the dissipation of activity-dependent increases in $[K^+]_o$ in rat optic nerve[9], rat vagus nerve[15], guinea-pig olfactory cortex (studied at 25 °C[5]) and rat brainstem[23]. This result is presumed to be a consequence of inhibition of active K^+ reuptake into neurons and glial cells, and perhaps blood vessels, mediated by activation of the Na^+ pump (see ref. 43). Application of 1 mM ouabain to the sciatic nerve in two experiments did not significantly change the rate of dissipation of activity-dependent increase in $[K^+]_o$ (not illustrated), although it caused a gradual deterioration in nerve excitability that presumably was due to a progressive loss of axonal ion gradients.

Another method of altering metabolically-dependent processes is via O_2 deprivation. Active K^+ removal in frog tectum[43] and in cat brain[22] is slowed in this manner. The effects of anoxia on K^+ removal in the sciatic nerve were examined. Anoxia was achieved by switching the nerve's atmosphere from 95% O_2/5% CO_2 to 95% N_2/5% CO_2. Anoxia produced a reversible increase in the baseline level of $[K^+]_o$ that averaged 1.8 mM ($n = 2$); the new steady-state $[K^+]_o$ was achieved about 20 min after the start of anoxia. Although the evoked increases in $[K^+]_o$ were larger under anoxic conditions, there was no

prolongation of K^+ removal (Fig. 7). The results of experiments with ouabain, temperature variation and anoxia were consistent with the conclusion that, in rat sciatic nerve, the removal of K+ after stimulation-dependent accumulation was not an active process.

DISCUSSION

The above studies demonstrate the degree to which activity-dependent K^+ accumulation occurs in a representative mammalian peripheral nerve. The magnitude of this accumulation is slight at 37 °C, in comparison to the CNS where increases to 10–12 mM may be achieved with intense stimulation[9,31,45]. This difference is likely to be a consequence of several factors. The amount of extracellular space (ECS) surrounding peripheral axons is greater than is the case in the CNS[36] and this directly influences the extent of ion accumulation (see ref. 31). Methods for determining the absolute volume of the ECS are imprecise and results may vary depending on the brain region assessed. Nevertheless, the ECS appears to occupy about 12–20% of total brain volume[27,32]; ECS volume in the peripheral nervous system appears to be about 22–30%[42]. Tortuosity of diffusion path can also play a role in determining the magnitude of K^+ accumulation in nervous tissue[32]. Although this parameter has only been analyzed in the CNS[32], intuitively it is likely to be less important in peripheral nerve where ECS volume fraction is larger. In the CNS, ECS volume is not a constant, but can rapidly and reversibly decrease with neural activity[11,40]. This activity-dependent decrease in ECS volume can magnify the amplitude of extracellular ion accumulation in the brain, but peripheral nerve does not exhibit significant ECS shrinkage with stimulation (Ransom and Yamate, unpublished observations).

The extent of K^+ accumulation also depends on the efflux of K^+ from neural tissue as has been demonstrated in the rat optic nerve during development[9]. In the case of peripheral nerve, this efflux of K^+ was presumably from active axons. Active K^+ channels are known to exist in these axons[13] but it is possible that there are quantitative differences in the magnitude of K^+ efflux from PNS and CNS axons; if CNS axons contributed more extracellular K^+ during activity than PNS axons, this would contribute to the differences in magnitude of activity-dependent K^+ accumulation.

Alteration in K^+ efflux from stimulated axons may explain the effect of temperature on K^+ accumulation in sciatic nerve. The increased amount of K^+ accumulation seen at 22 °C (compared to 36 °C) may result from prolonged current flow from K^+ channels whose inactivation time constant has been increased at the cooler temperature[35]. This temperature effect was probably not the result of decreased K^+ removal because the apparent rate of K^+ removal was not markedly altered by this range of temperature change (see below).

The amplitude of stimulated increases in $[K^+]_o$ in peripheral axons might be underestimated by the methods employed in this study (the same is also true of most studies carried out in the CNS). There is some reason to believe, based on optical studies using voltage-sensitive dyes, that axonal discharge causes K^+ to differentially accumulate in a highly restricted space between paranodal loops and the active nodal membrane[24]; the accurate magnitude and time course of K^+ accumulation in this special location cannot be measured by conventional ion-sensitive electrodes inserted into the ECS, but the magnitude is likely to be greater, perhaps much greater, than in the general ECS.

The removal of K^+ after activity-dependent accumulation of this ion was remarkably slow in rat sciatic nerve in comparison to the rate of K^+ removal in rat CNS (e.g. ref. 9; see also refs. 31, 45). The high rate of removal of accumulated extracellular K^+ within the mammalian CNS is the result of active reuptake into other compartments[5,8,9,17,26,45,47,48], although specific passive reuptake mechanisms also participate[21,30]. The relative contributions of neurons and glial cells, and perhaps even capillary endothelial cells, to K^+ removal remains undetermined[38]. The absence of active K^+ reuptake appeared to underlie the slow rate of extracellular K^+ removal in peripheral nerve. Large changes in temperature, in a range that dramatically altered extracellular K^+ reuptake in cat cortex[26], had minimal effect on K^+ removal in the sciatic nerve. Other manipulations that can alter active K^+ reuptake, namely the application of ouabain and periods of anoxia, likewise failed to alter the rate of K^+ removal in the sciatic nerve.

Passive mechanisms of K^+ removal include diffusion, spatial buffering and passive KCl uptake[5,21,30,34]. Blockade of K^+ channels should inhibit the latter two mechanisms[5,20]. Application of the K^+ channel blocker Ba^{2+}, however, did not significantly change the rate of K^+ removal in the sciatic nerve suggesting that diffusion was the primary mechanism for the dissipation of activity-dependent K^+ accumulation in this structure. Since the absolute amounts of measurable extracellular K^+ that accumulate in the nerve were small, especially at 37 °C, one can speculate that specialized K^+ removal mechanisms are simply unnecessary and their absence is an energy-saving practical adaptation. Ultimately, the axons that lose K^+ to the ECS during activity must recover an equal amount of K^+ from the ECS. The rate of this process, which is probably accomplished by the axonal Na^+ pump, is unknown but may be even slower than the pace of K^+ decline in the ECS after an

activity-induced build-up.

Peripheral nerve has a regulated microenvironment as a result of the blood–nerve barrier; this is composed of blood vessels lined with a continuous endothelium with intercellular tight junctions and a cellular sheath around nerve fascicules, the perineurium, that acts as a diffusion barrier[41]. The K^+ that accumulates around active nerves would not be able to freely diffuse into the lumens of blood vessels or across the perineurial sheath. Furthermore, at least in frog sciatic nerve, there is no evidence that K^+ is actively transported by either the perineurium or nerve blood vessels[41]. Thus, the blood–nerve barrier in rat sciatic nerve probably makes K^+ dissipation even slower than would be expected of a purely diffusional process.

The behavior of activity-dependent changes in extracellular ions in the sciatic nerve is somewhat different from what has been reported for rat vagus nerve and spinal root[6,12]. In rat vagus nerve, stimulation results in acid shifts in pH_o, and the magnitude of K^+ accumulation and its rate of dissipation are much greater than in sciatic nerve[12,15]; these differences may be because the vagus is primarily composed of small unmyelinated fibers while the rat sciatic nerve contains large myelinated fibers. In contrast to large myelinated fibers that conduct in saltatory fashion, small unmyelinated fibers are more subject to activity-dependent increases in intracellular Na^+ concentration that would stimulate the Na^+ pump[37]; high rates of Na^+ pump activity would assist in removal of accumulated extracellular K^+. Consistent with this idea, the removal of K^+ was slowed by exposure to ouabain and lowering the ambient temperature in the rat vagus nerve[15]; opposite results were seen here. As was the case in sciatic nerve, stimulation of rat spinal roots failed to elicit measurable changes in pH_o. The rate of K^+ removal in spinal roots[6], however, was much more rapid than in sciatic nerve. Since both of these nerves are predominately myelinated, the explanation for the difference in K^+ removal kinetics is not clear.

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