Activity-Dependent K^+ Accumulation in the Developing Rat Optic Nerve

Abstract. *Potassium-sensitive microelectrodes were used to study activity-dependent changes of extracellular potassium ion concentration ($[K^+]_o$) in rat optic nerves of different postnatal ages (1 day to adulthood). The maximum level to which $[K^+]_o$ rose with optimal frequencies of stimulation depended on age: mean maximum evoked $[K^+]_o$ was 17.2 mM in 1- to 3-day-old optic nerves and 9.8 mM in adult nerves. The ceiling $[K^+]_o$ seen in immature optic nerves, which is uniquely large for a mammalian central nervous system structure, may result from a relatively enhanced rate of evoked K^+ release.*

Neuronal activity in the presence of a restricted extracellular space typically results in a transient accumulation of excess extracellular K^+ (*1*). Studies on mammalian central nervous systems (CNS) indicate the existence of a remarkably constant "ceiling" level of extracellular K^+ concentration ($[K^+]_o$) of 10 m*M* to 12 m*M* (*2*), which is exceeded only under conditions of anoxia or spreading depression (*3*). Mechanisms that might help to determine this maximum $[K^+]_o$ include active K^+ reuptake, specific neuronal and glial membrane properties, the rate of neuronal K^+ release, tissue geometry, and intercellular coupling (*4*). Changes in the degree of K^+ accumulation with changes in tissue maturity have not yet been systematically considered [but see (*5*)], and may have important implications for developmental neurobiology as well as improve our understanding of K^+ homeostasis within the brain. We report here the ontogeny of activity-dependent K^+ accumulation as studied in a relatively simple structure of the mammalian CNS, the rat optic nerve. Although the axons of this nerve are functional at all ages, electron microscopy indicates that they are completely unmyelinated at birth, begin myelinating 6 to 8 days after birth, and are entirely myelinated in the adult (*6*). Neonatal nerves exhibit a maximum $[K^+]_o$ uniquely high among mammalian CNS structures; this concentration falls rapidly with maturation. We have investigated some of the possible mechanisms for this developmental change.

Microelectrodes sensitive to K^+, prepared in a conventional fashion, were used to record $[K^+]_o$ (*7*). Optic nerves from pigmented Long-Evans rats 1 day old to adulthood were dissected free with the arachnoid ensheathment intact, placed in a recording chamber, and bathed in an oxygenated physiological saline with 5 m*M* $[K^+]$ (*8*). The K^+-sensitive microelectrodes were positioned toward the center of each nerve and adjusted to give the maximum field potential (recorded through the indifferent barrel of the electrode assembly). Variable periods and frequencies of stimulation were applied to nerves by suction electrodes at current levels set at intensities that were twice those producing maximum field responses (supramaximal stimuli) and sufficiently long to activate the slowest fiber groups at each age (20 to 200 μsec).

Stimulation raised $[K^+]_o$ to a point that depended on stimulus frequency and the age of the animal. In nerves less than 3 days old, low frequencies of stimulation (1 to 5 Hz) significantly raised $[K^+]_o$ (Fig. 1A). The rate of accumulation and maximum $[K^+]_o$ progressively increased with higher stimulus frequencies, reaching 20 m*M* to 21 m*M* in some nerves at 10 to 20 Hz. Older nerves exhibited much lower $[K^+]_o$ (maximum of 11 m*M* in adults), even with stimulus frequencies as high as 400 Hz (Fig. 1B). The dependence of maximum evoked $[K^+]_o$ on stimulus frequency and optic nerve age is shown in Fig. 1C for four representative nerves. By varying stimulus frequency, the absolute maximum evokable $[K^+]_o$ was determined for 32 individual nerves of different ages. The major decrease in $[K^+]_o$ ceiling occurred within the first week of life (Fig. 1D), before myelination had begun (*6*).

We tested the possibility that major changes in the rate of K^+ dissipation might account for developmental variations in K^+ accumulation. After the stimulus train ended, $[K^+]_o$ fell toward the ambient concentration of 5 m*M* and usually dipped below this level, producing a period of undershoot in $[K^+]_o$ (Fig. $2A_1$), which increased in magnitude as the frequency or duration (or both) of the stimulus train was increased (Fig. $2A_2$). Such undershoots usually imply the existence of an active K^+ reuptake mechanism (*9*). There were no consistent differences between the undershoots seen in immature and mature optic nerves after comparable increases in $[K^+]_o$ (Fig. $2A_1$). The average rate of dissipation of comparable concentrations of accumulated K^+ (Fig. 2B) was slightly greater for immature than mature nerves (*10*). In optic nerves of all ages the kinetics of dissipation were dramatically slowed

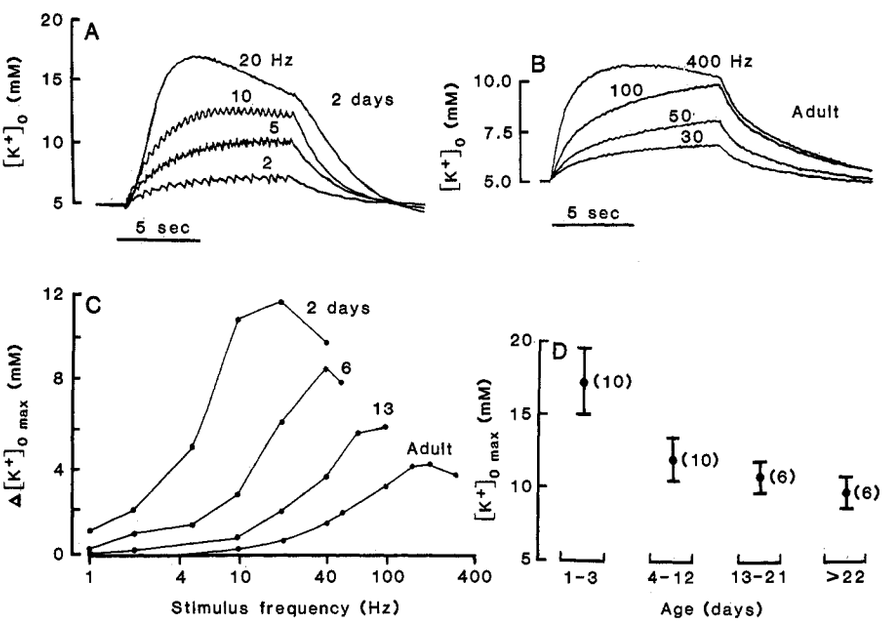

Fig. 1. (A) Evoked changes of $[K^+]_o$ in 2-day-old optic nerve. Ten-second trains of supramaximal stimuli were applied at the frequencies noted on each superimposed trace. Data were recorded on magnetic tape, digitized, and linearized by computer. (B) Evoked changes of $[K^+]_o$ in adult optic nerve. Stimulation procedure as in (A). Note change in $[K^+]_o$ scale. (C) Peak change in $[K^+]_o$ produced by 10-second trains of various frequencies applied to nerves of different ages. (D) Graph of the maximum activity-induced $[K^+]_o$ [derived from experiments of the type shown in (C)] as a function of age. Each point represents the mean ± standard deviation of grouped data; *N* is shown in parentheses. The difference between the two youngest groups is significant ($t = 5.92$, $P < .01$).

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and undershoots abolished by the addition of 50 μM strophanthidin (an inhibitor of Na^+- and K^+-dependent adenosinetriphosphatase) to the bathing medium (Fig. 2C). These observations indicate that active K^+ reuptake occurs in both mature and immature optic nerves and that age-related differences in maximum $[K^+]_o$ cannot be explained by a developmental change in reuptake mechanisms.

Intense neural activity alters the size of the extracellular space of mammalian brain (*11*). We reasoned that developmental changes in the amount of activity-dependent shrinkage of the extracellular space might account for age-dependent changes in maximum $[K^+]_o$. To test this possibility, we added 10 mM choline to the bath and allowed it to equilibrate with the nerves. Because of its high selectivity for quaternary amines, the K^+ electrode responds only to changes in extracellular choline concentration ($[Ch^+]_o$) under these conditions (*12*). Since Ch^+ does not easily cross cell membranes, changes in $[Ch^+]_o$ primarily reflect changes in the dimensions of the extracellular space. In nerves less than 5 days old, periods of intense stimulation produced minimum changes in $[Ch^+]_o$ (< 5 percent), whereas similar periods of stimulation in older nerves increased $[Ch^+]_o$ as much as 30 percent (Fig. 2D). Thus, the observed developmental sequence of the activity-dependent changes in extracellular space were in a direction opposite to that anticipated in order to explain the higher K^+ accumulation seen in young nerves.

When we increased $[K^+]_o$ in the bathing solution, there was a graded reduction in the change of $[K^+]_o$ evoked by a standard stimulus train. At bath $[K^+]_o$'s of between 16 mM and 20 mM, field potentials and changes in $[K^+]_o$ disappeared. Mature nerves failed at bath $[K^+]_o$'s that were only about 2 mM less than concentrations causing failure in immature nerves. Self-inhibition by high evoked $[K^+]_o$ may be a major determinant of the ceiling in neonatal nerves, but it cannot account for the much lower ceiling in adult nerves.

The extent of K^+ accumulation must also depend upon the rate at which K^+ is released by activated axons. The increase in $[K^+]_o$ resulting from individual supramaximal stimuli in immature nerves was about seven times that in adult nerves (inset, Fig. 2E). The developmental time course of $[K^+]_o$ changes resulting from single stimuli was similar to the time course observed for maximum $[K^+]_o$ accumulation (compare Fig. 1D with Fig. 2E) (when data from all

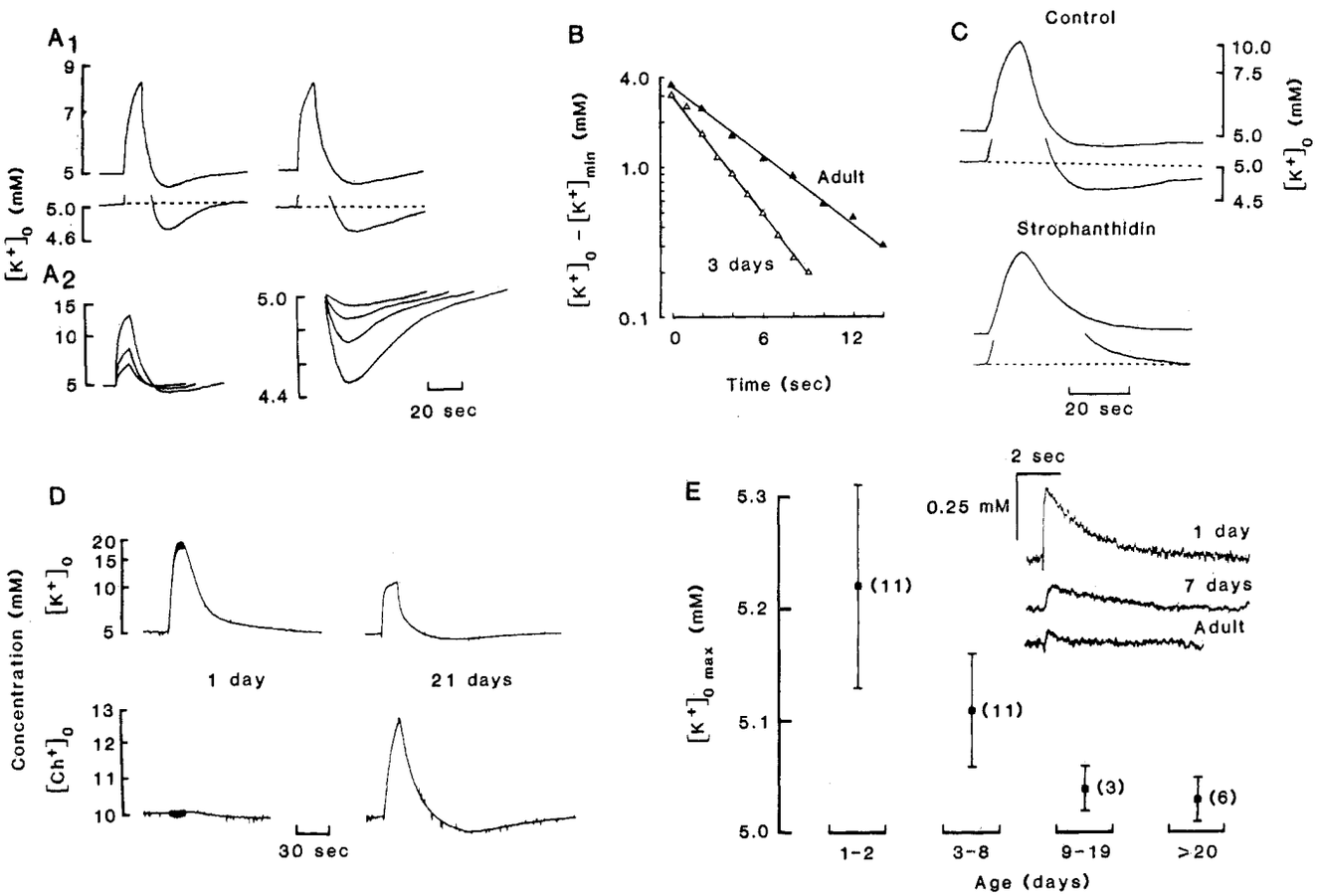

Fig. 2. (A_1) Poststimulus undershoots of $[K^+]_o$ induced by 10-second trains in a 2-day (5 Hz, left) and adult (50 Hz, right) nerve at low (top trace) and high (bottom trace) gain. (A_2) Superimposed traces at left are from a 3-day nerve stimulated at 5, 10, and 20 Hz. At right the undershoots from this nerve (at 2, 5, 10, and 20 Hz) have been superimposed and are shown at higher gain. (B) Kinetics of $[K^+]_o$ decay after an evoked increase. Stimulus frequencies have been adjusted to give comparable increases in $[K^+]_o$ (5 Hz for 3 days, 200 Hz for adult), and poststimulus decay of $[K^+]_o$ has been plotted on a semilogarithmic scale. $[K^+]_{min}$ is the lowest value to which $[K^+]_o$ falls after an evoked increase. (C) Strophanthidin (50 μM) block of poststimulus undershoot. Data from a 7-day nerve stimulated at 25 Hz for 10 seconds before and 10 minutes after introduction of strophanthidin to bath. Each $[K^+]_o$ transient is shown at low (top trace) and high (bottom trace) gains. (D) Activity-induced changes in extracellular volume. Top traces illustrate $[K^+]_o$ changes elicited by 10-second trains applied to 1-day (10 Hz) and 21-day (100 Hz) nerves in control solution. Bottom traces show measurements of $[Ch^+]_o$ after introduction of 10 mM Ch^+ to bathing solution. (E) Peak $[K^+]_o$ after single supramaximal stimuli, as a function of age. Each point is mean ± standard deviation derived from the number of nerves shown in parentheses. The difference between the two youngest groups is significant ($t = 3.49$, $P < .01$). Inset shows specimen records of $[K^+]_o$ changes following single shocks to three nerves of different ages.

ages were pooled, $r = .8$, $P < .001$). At similar stimulation frequencies, therefore, $[K^+]_o$ increased more rapidly in immature nerves than in mature nerves (Fig. 1, A and B). Mature nerves, however, exhibited high maximum rates of K^+ accumulation because they tolerated higher frequencies of stimulation than immature nerves (*13*), but in spite of this compensating factor the measured maximum rates of K^+ accumulation in adults were never more than 60 percent of the maximum rates in neonates. Moreover, during stimulus trains at highest frequencies, the high initial rates of K^+ accumulation in adults were maintained more briefly than those of neonatal nerves (Fig. 1, A and B).

The following observations may be relevant to the higher evoked $[K^+]_o$ seen in immature rat optic nerves. (i) Active K^+ reuptake is present in optic nerves of all ages, and the decline of evoked $[K^+]_o$ in immature nerves is at least as rapid as in adults. (ii) Activity-evoked shrinkage of the extracellular space is prominent only in mature optic nerves. (iii) Complete inhibition of evoked $[K^+]_o$ increase occurs at bath $[K^+]_o$'s of 16 m*M* to 20 m*M* for nerves of all ages. (iv) The rise in $[K^+]_o$ produced by a single stimulus is much larger in neonatal than in adult nerves. (v) Maximum rates of K^+ accumulation are greater and persist longer in neonates than in adults regardless of stimulus frequency. Thus, the high levels of evoked $[K^+]_o$ seen in immature animals seem to result from higher and more sustained rates of $[K^+]_o$ release, which come into equilibrium with K^+ dissipation rates at higher absolute $[K^+]_o$ than in adults. With maturation the maximum rate of K^+ accumulation declines, in part because the increase in $[K^+]_o$ per impulse becomes less. The inability of older nerves to sustain high rates of K^+ release may result from frequency-dependent conduction block. Although in some axons this process has been attributed to accumulation of extracellular K^+, our data suggest that other mechanisms are also prominent in the adult optic nerve [compare with (*14*)].

The maturational changes in $[K^+]_o$ evoked by a single stimulus occur primarily within the first postnatal week (Figs. 1D and 2E), before myelination has begun (*6*). These may reflect alterations in specific membrane properties or a topographical reorganization of ionic channels in anticipation of myelination. The existence of voltage-sensitive K^+ channels in mammalian myelinated nerves has been questioned (*15*), and active K^+ channels might become less prominent as the optic nerve matures (*6*). The absence of active K^+ channels would not necessarily reduce K^+ efflux, however, since this ion might still carry repolarizing current through K^+-specific leakage channels (*16*). On the other hand, our calculations suggest that axonal growth may reduce the density of excitable membrane per unit volume of nerve during the first week, solely accounting for the lower net increase in $[K^+]_o$ per impulse without invoking specific membrane changes (*17*).

Whether the enhanced K^+ accumulation seen with neural activity in immature optic nerves has more general significance for the mammalian CNS is not known. If this maturational sequence proves to be widespread, the optic nerve may provide a conveniently simple experimental system for further analysis.

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8. The standard perfusion solution contained NaCl, 124 m*M*; KCl, 5 m*M*; NaH_2PO_4, 1.25 m*M*; $MgSO_4$, 2 m*M*; $CaCl_2$, 2 m*M*; $NaHCO_3$, 26 m*M*; and dextrose, 10 m*M*, saturated with 95 percent O_2 and 5 percent CO_2. All experiments were done at 37°C. When stimulation experiments were repeated at bath $[K^+]_o$ of 3 m*M*, the absolute maximum concentrations of evoked $[K^+]_o$ were unchanged.
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10. The half-time ($t_{1/2}$) of $[K^+]_o$ decrease was measured in six immature nerves (< 5 days) and six mature nerves (> 20 days) after a 10-second period of repetitive stimulation. Since the magnitude of $[K^+]_o$ accumulation may influence the rate of fall, we compared only $[K^+]_o$ responses which were of similar size (that is, the average peak $[K^+]_o$ response of the six immature nerves was not significantly different from that of the six mature nerves, $P > .1$). Mean $t_{1/2}$ was 3.1 seconds for the immature nerves and 4.8 seconds for the mature nerves [t (10) = 2.59, $P < .05$].
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13. Immature nerves were unable to follow stimulus frequencies beyond 40 Hz, whereas adult nerves could usually follow at 300 Hz. On the basis of average amounts of K^+ accumulation per impulse (0.22 m*M* per impulse for immature nerves, 0.03 m*M* per impulse for adult), therefore, the theoretical maximum rates of K^+ accumulation would be 8.8 m*M*/sec and 9 m*M*/sec for immature and adult nerves, respectively.
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17. Using the largest single stimulus $[K^+]_o$ changes at each age within the first postnatal week and the measured axonal geometries (*6*), we have calculated an approximate rate of membrane K^+ efflux of 0.7 to 0.9 pmole/cm^2 per impulse, regardless of age. Though subject to uncertainties of extracellular volume and axon measurement, these estimates compare favorably with measurements in unmyelinated rabbit vagus nerves: 1 pmole/cm^2 per impulse [R. D. Keynes and J. M. Ritchie, *J. Physiol. (London)* **179**, 333 (1965)]. Because glia are largely undifferentiated and still proliferating during the first week [R. D. Skoff, D. L. Prince, A. Stocks, *J. Comp. Neurol.* **169**, 291 (1976); *ibid.*, p. 313], it is unlikely that our results are due to the development of a K^+ diffusion barrier shielding the electrode from accurate measurement of periaxonal $[K^+]_o$.
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