Axon Conduction and Survival in CNS White Matter During Energy Deprivation: A Developmental Study

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Fern, Robert, Peter Davis, Stephen G. Waxman, and Bruce R. Ransom. Axon conduction and survival in CNS white matter during energy deprivation: a developmental study. J. Neurophysiol. 79: 95–105, 1998. We investigated the postnatal development of axon sensitivity to the withdrawal of oxygen, glucose, or the combined withdrawal of oxygen + glucose in the isolated rat optic nerve (a CNS white matter tract). Removal of either oxygen or glucose for 60 min resulted in irreversible injury in optic nerves from adult rats, assessed by loss of the evoked compound action potential (CAP). Optic nerves at ages <P10 showed no permanent loss of function. CAP sensitivity to the withdrawal of oxygen or glucose emerged during a critical period in development between postnatal days 10–20 (P10–P20). The CAP was unchanged in adult optic nerve for 45 min after the withdrawal of glucose, demonstrating the presence of a significant energy reserve. Periods of glucose withdrawal >45 min caused the selective loss of late CAP components; this was not seen with oxygen deprivation. The amplitude of the early component recovered to 94.8% of control after 60 min of glucose withdrawal, although total CAP area recovered to only 42.3%. Combined oxygen + glucose withdrawal for 60 min produced a greater degree of permanent CAP loss than 60 min of glucose or oxygen withdrawal individually in optic nerves from rats older than P4. Younger than P4 optic nerves showed no permanent loss of function from 60 min of combined oxygen + glucose withdrawal. Unexpectedly, optic nerves from P21–P49 rats recovered significantly less after all three conditions than adult optic nerves (>P50). It is probable that this period of final myelination corresponds to a time of heightened metabolic activity in white matter. The tolerance of CNS white matter to energy deprivation can be categorized into four stages that are correlated with specific developmental features: premyelination (P0–P4), highly tolerant to anoxia, aglycemia and combined anoxia/aglycemia; early myelination (P5–P20), partially tolerant of anoxia and aglycemia but not to combined anoxia/aglycemia; late myelination (P21–P49), very low tolerance of anoxia, aglycemia and combined anoxia/aglycemia; and mature (>P50), low tolerance of anoxia, aglycemia and combined anoxia/aglycemia. The relative resistance of optic nerve function to glucose withdrawal in the presence of oxygen, compared with glucose withdrawal in the absence of oxygen, is presumably due to the presence of oxygen-dependent energy reserves such as astrocytic glycogen, amino acids, and phospholipids.

INTRODUCTION

The CNS of the neonatal mammal, compared with the adult, is resistant to anoxia and ischemia (Duffy et al. 1975; Kabat 1940). This is highly advantageous to the neonate because brain perfusion is commonly subject to interruption in the pre- to postnatal periods (Armstrong 1993; Peneth et al. 1994; Vannucci 1990; Volpe 1992). Prolonged neonatal anoxia-ischemia, however, can result in extensive CNS injury, and white matter structures within the CNS are selectively vulnerable (Paneth et al. 1994). This characteristic pattern of injury, termed periventricular leukomalacia (Banker and Larroche 1962), is the most common neurological lesion associated with cerebral palsy, a disorder that affects between 1.5 and 2.5 per 1,000 live births a year (Armstrong 1993; Kuban and Leviton 1994; Volpe 1992). Periventricular leukomalacia may occur before, during, or after the onset of myelination (Paneth et al. 1994), although the relationship between the stages in white matter development and the predilection to injury is not known. We have investigated the onset, extent, and recovery of injury during three forms of energy deprivation experienced by the neonatal CNS (anoxia, glucose withdrawal, and combined anoxia + glucose withdrawal), using the isolated rat optic nerve. Because the CNS of the rat is immature at birth (Davison and Dobbing 1966; Romijn et al. 1991), this preparation can be used to study injury before myelination, during the early stage of myelination when axons receive their first wraps of myelin, and during the late stage when the rate of myelin deposition is at its peak (Foster et al. 1982; Skoff et al. 1976). We expected that studying the effects of anoxia and glucose withdrawal and combined anoxia + glucose withdrawal would provide information about the capacity of white matter to tolerate energy deprivation and permit analysis of the contribution of energy reserves to level of tolerance at key points in development.

METHODS

The in vitro rat optic nerve preparation (Davis and Ransom 1987; Stys et al. 1992) was used to study the effects of anoxia, zero-glucose, and combined zero-glucose/anoxia on CNS white matter function during postnatal development. The optic nerve is a CNS white matter tract, consisting of astrocytes and CNS axons myelinated by oligodendrocytes (Butt and Ransom 1993; Forrester and Peters 1967; Foster et al. 1982). The optic nerve has the practical advantage of allowing reproducible electrophysiological recordings, which provide a quantitative measure of the capacity of a CNS white matter tract to conduct action potentials. The developmental features of optic nerve morphology, electrophysiology, and cell (glial) differentiation have been well characterized (Butt and Ransom 1993; Foster et al. 1982; Raff and Miller 1984), and the mechanisms underlying axonic injury in the optic nerve have been analyzed in detail (Fern et al. 1995; Ransom et al. 1993; Stys et al. 1992). This preparation has the additional advantage that the postnatal development of the rat CNS corresponds develop-
mentally to the prenatal (<P12/P13), term (P12/P13), and postnatal (>P12/P13) human infant (Davison and Dobbing 1966; Romijn et al. 1991), providing a model for the study of the relevant developmental epochs in the human.

Long-Evans rats between P0 and P2 were anesthetized by hypothermia and P3–P86 rats were anesthetized with an 80% CO₂-20% O₂ gas mixture before decapitation. The optic nerves were dissected free into cold artificial cerebrospinal fluid (ACSF); the time between decapitation and cooling was ~45 s. Nerves then were placed in a modified interface perfusion chamber (Medical Systems, Greenvale, NY) and incubated for 60–90 min before measurements were initiated. Optic nerves were maintained at 37°C, oxygenated in a 95% O₂-5% CO₂ atmosphere, and perfused with ACSF with a pH of 7.45. Unless otherwise noted, the ACSF contained (in mM) 153 Na⁺, 3 K⁺, 2 Mg²⁺, 2 Ca²⁺, 133 Cl⁻, 26 HCO₃⁻, 2 PO₄⁻, and 10 glucose.

**Adult optic nerves**

Two techniques were employed for recording evoked compound action potentials (CAP) from optic nerves taken from >P20 rats. In both cases the distal end of the optic nerve was positioned within an ACSF-filled glass stimulating electrode and CAPs were evoked by a 125% supramaximal stimulus (50-μs duration). CAPs were recorded via a second ACSF-filled glass electrode positioned around the proximal nerve end. The area under the CAP subsequently was calculated by computer. In the majority of cases, the electrodes were left in place throughout the course of the experiment. It was found that stable recordings could be achieved provided that a small gap was left between the optic nerve and the internal diameter of the recording electrode. This allowed any slow depletion of the electrode filling solution to occur without disturbing the position of the optic nerve. The impedance across the recording electrode was monitored and remained constant under these conditions. Some recordings were obtained using the method of Stys et al. (1991), which compensates for changes in the impedance across the recording electrode and allows removal and repositioning of the electrodes during the experiment. No differences were found between the two slightly different recording techniques.

Anoxia was induced by changing to a 5% CO₂-95% N₂ atmosphere (Stys et al. 1990). Zero-glucose was induced by initiating a period of perfusion with glucose-free ACSF. Combined zero-glucose/anoxia was produced by switching to glucose-free ACSF and 5% CO₂-95% N₂. The presence of glucose in the electrode filling solution was found to significantly increase the ability of the optic nerves to tolerate perfusion with zero-glucose ACSF; and glucose therefore was omitted from the electrode filling solution in these experiments. CAP recovery after an insult was determined by measuring CAP area 60 min after restoring control conditions, a period that allowed maximal recovery to occur (see Results). Changes in the area under the CAP were assumed to correspond to changes in the number of axons capable of conducting action potentials along the optic nerve (Cummins et al. 1979; Stys et al. 1991; Wijesinghe et al. 1991).

**Neonatal optic nerves**

CAPs recorded from nerves taken from animals younger than P20 are biphasic (Foster et al. 1982), which makes the measurement of CAP area problematic. Furthermore, long-duration stimulus pulses (between 100- and 600-μs) were required to elicit supramaximal responses in neonatal optic nerves, which occasionally produced slow deflections in the baseline. CAP amplitude (measured between the peak of the largest positive component and the peak of the largest negative component) therefore was used instead of CAP area in recordings from optic nerves from <P20 rats. Electrodes were left in place throughout the experiments and anoxia, zero-glucose and joint zero-glucose/anoxia conditions were produced as described for adult optic nerves.

**Brain slices**

Standard techniques for preparing and maintaining neocortical slices were employed for comparisons of gray matter and white matter, (e.g., Connors et al. 1982). Briefly, slices of adult rat frontal-parietal cortex were prepared. Glass pipettes (10-μm tips), filled with normal saline, were used to record cortical field potentials from layers 2 or 3, evoked by stimulating afferent fibers from the underlying white matter with a separate electrode. Recording conditions, gas mixtures, and solutions were as described for optic nerve experiments.

**Statistics**

Results are reported as means ± SD. Statistical significance was determined by Student’s t-test or analysis of variance as appropriate.

**RESULTS**

**Anoxia**

Under conditions of constant glucose (10 mM), anoxia rapidly caused the loss of the CAP in adult optic nerves (Fig. 1A). CAP area approached zero after 360 s of anoxia, and all three components of the CAP were lost at a similar rate (Fig. 1A, inset). We directly compared the effects of anoxia on nerve conduction and synaptic transmission using the adult optic nerve and neocortical slice, employing the same perfusion chamber, flow rate, solution, and gas mixtures for both preparations. Extracellular synaptic potentials were recorded from layers 2 and 3 of frontal-parietal cortical slices after stimulation of the underlying white matter (Fig. 1D). After the onset of anoxia, synaptic potentials were lost at a similar rate (Fig. 1B) to the loss of the optic nerve CAP (Fig. 1A), and no synaptic potential was evident after 270 s of anoxia (n = 2). Although the optic nerve CAP recovered almost fully after a 15-min period of anoxia (Fig. 1C), no recovery of synaptic potentials was found after an anoxic period of the same duration (Fig. 1D). This is consistent with the large body of evidence indicating that the mechanisms of white matter and gray matter anoxic injury are very different (Cherubini et al. 1989; Hansen 1985; Waxman et al. 1991).

The rate and the extent of CAP recovery from anoxia in the adult optic nerve were dependent on the length of the anoxic insult (Fig. 2). Recovery from a 5-min anoxic period was typically complete after 15–20 min of re-oxygenation, but 30–40 min of re-oxygenation was required before recovery reached a plateau after 60 min of anoxia (Fig. 2B). Short periods of anoxia were followed by almost complete recovery of CAP area, and the degree of CAP recovery was progressively lower after longer periods of anoxia (Fig. 2C). No recovery was found after periods of anoxia of ≥75 min. The shape of the postanoxic CAP was typically similar to that of the preanoxic CAP with all three components of the CAP being retained (Fig. 2A).

The development of the optic nerve’s sensitivity to anoxia is shown in Figs. 3 and 4. The effect of a standard 60-min period of anoxia was examined in optic nerves from rats of
loss of the CAP during a 60-min anoxic period and recovered to only a limited degree (Fig. 3, P31). At this developmental stage, the CAP had similar shape characteristics to the adult. The CAP recorded from adult optic nerves (>P50) usually recovered to a greater extent from a 60-min period of anoxia than did the CAP recorded from P21–P49 optic nerves (compare P31 and Adult recordings in Fig. 3).

CAP area (>P20) or amplitude (<P20) were monitored periodically during anoxia and re-oxygenation (Fig. 4A), and the extent of CAP recovery was plotted against postnatal age (Fig. 4B). The rate of decline of the CAP increased with age, but a relationship between age and rate of recovery after re-oxygenation was not apparent (Fig. 4A). Optic nerves can be grouped into four developmental stages according to their sensitivity to anoxia: 0≤P10, optic nerves were resistant to anoxia; between P10 and P20, optic nerves showed no systematic change in CAP during or after the period of anoxia (Fig. 3, P2); optic nerves from P11–P20 had CAPs with intermediate shape characteristics and showed an intermediate sensitivity to anoxia, undergoing partial conduction block during anoxia and recovering to less than the initial CAP amplitude after re-oxygenation (Fig. 3, P14). Optic nerves from P21–49 rats displayed complete recovery after 60 min of re-oxygenation. Brief periods of anoxia were followed by high levels of recovery while >75 min of anoxia resulted in no CAP recovery. Correlation coefficient of the linear regression line is r = 0.953.

Protocol for this experiment is shown schematically (inset), with the solid bars at the top indicating the length of the anoxic periods, which correspond to different degrees of CAP recovery (A–C).

FIG. 1. Effects of anoxia on nerve conduction and synaptic transmission in the adult CNS. A: plot showing the effects of anoxia on action potential conduction in the adult rat optic nerve monitored as the area under the compound action potential (CAP; n = 7). Inset: specimen records of CAPs recorded from an optic nerve at the indicated times (in min) after the onset of anoxia. All 3 components of the CAP decline at a similar rate. B: effects of anoxia on synaptic transmission monitored as the amplitude of the compound synaptic potential (CSP) recorded from rat neocortical brain slices (n = 2). Time courses of failure of synaptic transmission and of action potential conduction were similar. C: specimen records showing the CAP from an optic nerve before, during, and after a 15-min period of anoxia. D: specimen records showing the evoked CSP from a neocortical slice before, during, and after a 15-min period of anoxia. Note that this duration of anoxia had a largely temporary effect on action potential conduction (C) but had a permanent effect on synaptic transmission (D).

FIG. 2. CAP recovery after anoxia in adult optic nerve is a function of the duration of anoxia. A: specimen CAPs recorded before (Preanoxic) and 60 min after (Postanoxic) a 60-min period of anoxia. Note that the postanoxic CAP has 3 components, similar to the preanoxic CAP. B: rate and magnitude of recovery of CAP area are shown for 3 representative adult optic nerves subjected to 5, 25, or 60 min of anoxia. Magnitude and rate of recovery were inversely proportional to the duration of anoxia. Steady-state value of CAP recovery was achieved ~30–40 min after the conclusion of the anoxic period. C: relationship between recovery of CAP area and the duration of the period of anoxia. CAP recovery was measured after 60 min of reoxygenation. Brief periods of anoxia were followed by high levels of recovery while >75 min of anoxia resulted in no CAP recovery. Correlation coefficient of the linear regression line is r = 0.953.

Protocol for this experiment is shown schematically (inset), with the solid bars at the top indicating the length of the anoxic periods, which correspond to different degrees of CAP recovery (A–C).
FIG. 3. CAP recorded from immature optic nerve was resistant to anoxia-induced dysfunction and injury. Preanoxic CAP (Control), the CAP recorded at the end of 60 min of anoxia (Anoxia), and the CAP after 60 min of reoxygenation (Recovery), are shown for optic nerves from rats at 5 different ages. CAP recorded from P2 optic nerve was not affected by anoxia while the CAP from P14 optic nerve was partially lost during anoxia and did not recover fully after reoxygenation. Optic nerves showed progressively more irreversible injury with 60 min of anoxia. Note that recovery of the CAP from anoxia at P31 was less than observed in the adult optic nerve.

showed progressively more extensive and rapid conduction failure during anoxia, and recovered progressively less, between P21 and P45, optic nerves showed the greatest sensitivity to anoxia (mean recovery of CAP area between P21 and P45 = 24.7 ± 12.5%, n = 12; mean recovery of CAP area between P50 and P86 = 37.5 ± 11.1%, n = 41; P < 0.0001); in optic nerves between P45 and P86, the extent of recovery from anoxia did not vary systematically with postnatal age.

Zero glucose

In the presence of oxygen, perfusion with glucose-free ACSF did not result in any immediate change in the CAP in the adult optic nerve. CAP area remained largely unchanged for ~40 min in zero-glucose, at which point CAP area began to decline (Fig. 5A). The dashed curve in Fig. 5A is the mean rate of decline of CAP area during anoxia, highlighting the difference in the time of onset and rate of loss of CAP area in zero-glucose, CAPs were elicited at a rate of 1/min in both cases. CAP area reached 24.8 ± 7.4% (n = 13) of the initial CAP area 65 min after initiating a 60 min of perfusion with glucose-free ACSF (CAP area continued to decline for ~5 min after reperfusion with 10 mM glucose). The reduction in CAP area produced by zero-glucose, unlike that produced by anoxia (Fig. 1, inset), did not affect all components of the CAP equally (Fig. 5, B–D). The second and third components of the CAP were suppressed consistently before, and recovered less, than the early CAP component. The amplitudes of the first and second components of the CAP were monitored and are shown in Fig. 5, C and D, demonstrating that the amplitude of the second component was significantly reduced before any reduction in the first component was apparent. After 60 min in normal glucose, after 60 min of zero-glucose, the mean amplitude of the first CAP component recovered to 94.8 ± 11.3% of initial amplitude, whereas the mean amplitude of the second component recovered to 39.8 ± 21.5% (n = 13; P < 0.0001).

The degree of optic nerve injury produced by different periods of perfusion with zero-glucose was investigated. After perfusion with zero-glucose, the recovery of CAP area was found to be maximal after 60 min of reperfusion with ACSF containing 10 mM glucose (Fig. 6D). Recovery therefore was measured at this point. In the adult optic nerve, no significant loss of CAP area occurred after 45 min of zero-glucose (Fig. 6A), but after 65 min of zero-glucose, the CAP was irreversibly lost (Fig. 6B). When the extent of CAP recovery is plotted against the period of perfusion with zero-glucose, the development of irreversible injury between 45 and 65 min of perfusion with zero-glucose is shown clearly (Fig. 6C). This contrasts with the injury produced by anoxia that developed progressively during the anoxic period (Fig. 2C). Recovery after 0 min zero-glucose is not 100% because CAP area was lower.
ENERGY DEPRIVATION IN WHITE MATTER

Anoxia / zero-glucose of <15 min did not produce any permanent reduction in CAP area in the adult optic nerve (Fig. 9, A and B). Longer exposure did result in significant permanent reductions in CAP area, and no recovery was found after 65 min of combined oxygen + glucose withdrawal (Fig. 9B). The rates of recovery of CAP area were faster after shorter periods of combined zero-glucose/anoxia (Fig. 9A), and maximal recovery was evident after 60 min after 60 min of the ischemic-like conditions. The effects of a standard 60-min period of combined zero-glucose/anoxia were therefore assessed after 60 min of recovery. Zero-glucose anoxia + zero-glucose of <15 min did not produce any permanent reduction in CAP area in the adult optic nerve (Fig. 9, A and B). Longer exposure did result in significant permanent reductions in CAP area, and no recovery was found after 65 min of combined oxygen + glucose withdrawal (Fig. 9B). The rates of recovery of CAP area were faster after shorter periods of combined zero-glucose/anoxia (Fig. 9A), and maximal recovery was evident after 60 min after 60 min of the ischemic-like conditions.

The development of sensitivity to 60-min perfusion with zero-glucose in optic nerves from rats of various ages was similar to the development of sensitivity to anoxia (Figs. 7 and 8). The CAP from <P10 optic nerves was not significantly affected by glucose withdrawal (Fig. 7, P6; and Fig. 8), and sensitivity to zero-glucose developed between P11 and P20 (Fig. 7, P14; and Fig. 8). CAP area recovered significantly less between P21 and P49 than in optic nerves at >P50 (mean recovery of CAP area between P21 and P49 = 20.9 ± 10.0%, n = 12; mean recovery of CAP area from >P50 = 42.3 ± 10.0%, n = 12; P < 0.0001). The rates of decline of the CAPs recorded from neonatal rats (<P50) was greater than for CAPs recorded from adult optic nerves (>P50) after the withdrawal of glucose (Fig. 8A). There was no apparent difference in rates of CAP recovery (Fig. 8A).

Zero-glucose anoxia

During ischemia in the CNS, both glucose and oxygen supply are interrupted. We found that periods of combined

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**FIG. 4.** Effects of 60 min of anoxia on optic nerve CAPs recorded from rats of various ages. A: magnitude of the CAP recorded from rats of various ages is plotted against time after the initiation of a 60-min anoxic period. Anoxia had no effect on CAP amplitude in <P10 optic nerves, and CAP recovery was less at P31 than at P75. Rate of fall of the CAP was proportional to age, with the CAP being lost most rapidly from adult animals. B: degree of CAP recovery after 60 min of anoxia (measured after 60 min of reoxygenation) is shown as a function of postnatal age. Susceptibility to anoxic injury developed between P10 and P20, and P21–P44 optic nerves recovered less than >P45 optic nerves. Adult level of CAP susceptibility to anoxic injury was acquired by P45.

**FIG. 5.** Effects of glucose withdrawal on CAP area and shape in adult rats. A: mean CAP area is plotted against time after the initiation of perfusion with artificial cerebrospinal fluid (ACSF) containing no glucose (n = 13; ● —● —●). For comparison, the rate of CAP decline produced by anoxia is shown (— — — ; taken from Fig. 1A). Extent of recovery after 60 min of reperfusion with normal ACSF also is shown (○ at time = 120 min). B: representative recordings of the CAP recorded from an adult optic nerve during perfusion with zero-glucose ACSF. Second and 3rd peaks of the CAP are shown as a function of time during and after perfusion with zero-glucose for 60 min. D: summary graph showing the mean amplitudes of the 1st and 2nd peaks of CAPs from adult optic nerves perfused with zero-glucose ACSF (n = 13). Amplitudes are shown as percent of control amplitude. Second peak is more sensitive to the withdrawal of glucose than is the 1st peak. First CAP peak recovered to 94.8 ± 11.3% of initial amplitude, and the second CAP component recovered to 39.8 ± 21.5% (n = 13; P < 0.0001).
FIG. 6. Effect of different periods of perfusion with zero-glucose ACSF on CAPs recorded from adult optic nerves. A: CAP remaining 60 min after recovery from a 45-min period of perfusion with zero-glucose ACSF is shown superimposed on the control CAP. Postaglycemic CAP is shaded. Note that the CAP recovered almost completely. B: similar records to those shown in A but the period of perfusion with zero-glucose was 65 min. Under these conditions the CAP was irreversibly lost. C: extent of CAP recovery after different periods of perfusion with zero-glucose; note that permanent dysfunction was minimal for periods of glucose withdrawal <45 min. D: extent and rate of CAP recovery after exposure to 60 min of zero-glucose is shown as a function of time after return to normal glucose. Extent of CAP recovery reached a stable level after 30 min.

FIG. 7. CAP recorded from neonatal optic nerve was resistant to withdrawal of glucose in the presence of oxygen. Perfusion with zero-glucose ACSF had no effect on the CAP at P6 but reduced the CAP at P14. CAP recorded from P23 optic nerve recovered less than that recorded from adult optic nerve. Development of postnatal sensitivity of the optic nerve to dysfunction and injury during glucose withdrawal was similar to that found with anoxia (Fig. 3).

glucose/anoxia for 60 min reduced CAP area to a significantly greater extent than did either 60 min of anoxia or 60 min of glucose withdrawal alone (mean CAP recovery after combined zero-glucose/anoxia = 19.5 ± 8.6%, n = 9; compared with 37.5 ± 11.1%, n = 41 after 60 min anoxia, P < 0.0001; and 42.3 ± 10.0%, n = 13 after zero-glucose, P < 0.0001).

The development of sensitivity to 60 min of combined zero-glucose/anoxia was distinct from development of sensitivity to anoxia or zero-glucose individually (Figs. 10 and 11). Unlike the individual effects of anoxia or zero-glucose, combined zero-glucose/anoxia caused a reduction in CAP amplitude recorded from <P10 optic nerves (Figs. 10, P2 and P5, and 11A). CAP amplitude in P0–P4 optic nerves fell progressively during the 60 min of zero-glucose/anoxia, but after reperfusion with normal oxygenated ACSF, CAP amplitude recovered either fully or extensively (Figs. 10, P2, and 11B). The rate at which the CAP declined during zero-glucose/anoxia increased with age (Fig. 11A), and optic nerves from P5–P20 rats showed irreversible loss of the CAP of an extent similar to that found in optic nerves from adult rats (Figs. 10, P5 and P12, and 11B). As was the case for anoxia or zero-glucose exposure individually, optic nerves from P21–P49 rats showed significantly less recovery of CAP area than optic nerves from adult animals following combined zero-glucose/anoxia (mean recovery of CAP area in optic nerves from P21–P49 rats = 7.6 ± 3.4%, n = 6; mean CAP recovery in optic nerves from >P50 optic nerves =
The development of sensitivity to energy deprivation has not previously been studied in white matter. In rat gray matter, the sensitivity of synaptic transmission to anoxia develops progressively from birth to P21 (Cherubini et al. 1989), as does the sensitivity of gray matter to the withdrawal of glucose (Crépel et al. 1992). This contrasts with the development of sensitivity to oxygen or to glucose withdrawal in white matter that occurred rapidly between P10 and P20. The resistance of the neonatal CNS to energy deprivation is thought to be a consequence of the low metabolic rate of the immature CNS (Duffy et al. 1975; Hansen 1985; Thurston and McDougal 1969). Indeed, the metabolic rate of the CNS rises in step with the development of sensitivity to energy deprivation in gray matter (Cherubini et al. 1989; Chugani et al. 1991; Crépel et al. 1992; Nehlig et al. 1988). Unlike gray matter, the development of CNS white matter includes a period that is dominated by the process of myelination. In the rat optic nerve, some axons already have acquired a single layer of myelin by P6–P7 (Foster et al. 1982; Skoff et al. 1976). The proportion of axons that have at least a single layer of myelin increases from a few percent at P6 to 85% at P28, and all axons are ensheathed by adulthood (Foster et al. 1982; Skoff et al. 1976). Heightened metabolic activity associated with the anabolic process of myelination may underlie the rapid development of sensitivity to energy deprivation.

**FIG. 9.** CAP recovery from different periods of combined zero-glucose/anoxia in optic nerves from adult rats. A: plot of the rate and extent of CAP recovery after 5, 25, and 60 min of combined zero-glucose/anoxia. Recovery of the CAP was more rapid and more extensive after 5 min of zero-glucose/anoxia than after 60 min of this insult. Recovery after 60 min of zero-glucose/anoxia was maximal after 30–40 min. B: extent of CAP recovery is shown as a function of the period of exposure to zero-glucose/anoxia. CAP recovery was measured 60 min after the period of zero-glucose/anoxia. Note that short (5–10 min) periods of zero-glucose/anoxia have no significant permanent effect and that no CAP recovery was evident after 65 min of glucose + oxygen deprivation.

$19.5 \pm 8.6\%, n = 13; P < 0.001$. Compare Fig. 10, P23 and Adult.

**DISCUSSION**

The results allow the development of susceptibility to energy deprivation in CNS white matter to be delineated into four stages. Optic nerves from P0–P4 rats were highly resistant to all forms of metabolic insult tested. Optic nerves from this age group showed some CAP loss during combined oxygen/glucose withdrawal, but showed nearly full recovery after reperfusion with normal oxygenated ACSF. Optic nerves from P5–P20 rats were partially tolerant to oxygen withdrawal or glucose withdrawal individually but not to combined oxygen + glucose withdrawal. Optic nerves from P21–P49 rats had the lowest tolerance to all forms of energy deprivation. A stable adult level of tolerance to all three conditions was reached by P50.

**FIG. 10.** CAPs recorded from perinatal optic nerves were relatively resistant to 60 min exposure to zero-glucose/anoxia compared with the adult optic nerve CAP. Unlike anoxia or zero-glucose individually, 60 min of combined zero-glucose/anoxia produced a reduction in the CAP recorded from optic nerves from rats at <P10. Full CAP recovery was evident in P2 optic nerves after reperfusion with normal ACSF. Only partial recovery was observed at P5, and at P12, the extent of recovery was comparable with that in the adult. Very little recovery was observed at P23.
characteristics of the CNS change during development. In neonatal optic nerve axons, the Na⁺ channel density is very low, i.e., <2/μm² (Waxman et al. 1982). The density of Na⁺ channels increases in the CNS from birth to P21, followed by a significant decrease in the adult (Xia and Haddad 1994). The change in the density of Na⁺ channels is accompanied by a proportionate change in “persistent” Na⁺ current (Alzheimer et al. 1993), which is important to the development of anoxic injury in CNS white matter (Stys et al. 1993). Within the optic nerve, Na⁺ channels begin to aggregate in high density at the developing nodes of Ranvier during early myelination, between P11 and P20 (Waxman et al. 1982). That Na⁺ channel density is maximal at P21 is interesting considering the heightened sensitivity of white matter to energy deprivation observed at that age. In addition to changes in Na⁺ channels, neuronal Ca²⁺ currents also increase in magnitude in the postnatal period (Lorenzon and Foehring 1995), and it has been observed recently that Ca²⁺ channels also contribute to the development of anoxic injury in white matter (Fern et al. 1995).

Anoxia

The rate at which action potential conduction was lost during anoxia in adult optic nerves paralleled the rate at which synaptic transmission was lost during anoxia in neocortical slices, using the same equipment and protocol to induce anoxia in both preparations. Similar rates of loss of action potential conduction (Stys et al. 1992) and synaptic transmission (Cherubini et al. 1989; Hansen 1985) have been reported previously, but this is the first time that a direct comparison has been made. The relationship between the length of the anoxic period and the extent of postanoxic recovery in white matter was found to be monotonic, with longer periods of anoxia resulting in less recovery. It has been shown previously that the development of anoxic injury in white matter is dependent on Ca²⁺ influx and that deprivation in white matter as shown here (Azzerelli et al. 1980; Chugani et al. 1991; Davison and Dobbing 1966; Kennedy et al. 1972; Rice et al. 1981; Wiggins 1982). The rate at which optic nerve axons receive their first layer of myelin is maximal between P21 and P28 (Skoff et al. 1976), and it is from this point onward that deposition of myelin will be at its height. Our results show that this period in white matter development corresponds to a period of heightened sensitivity to energy deprivation.

The rate at which the CAP is lost during energy deprivation presumably will be related to the metabolic rate of the optic nerve because a high metabolic rate will determine how rapidly energy reserves are used. If the metabolic rate is high, energy reserves will be depleted rapidly and the CAP rapidly lost. Early loss of energy reserves during energy deprivation also should reduce CAP recovery. Given that rate of CAP decline and degree of CAP recovery should be related to a common variable, i.e., metabolic rate, they should be related to one another. This expectation was tested in Fig. 12, where the extent of CAP recovery is plotted against the half-time of CAP failure. Indeed, these variables are related, rapid CAP decline is associated with a low level of CAP recovery.

In addition to developmental changes in the energy requirements of white matter, several important molecular characteristics of the CNS change during development. In neonatal optic nerve axons, the Na⁺ channel density is very low, i.e., <2/μm² (Waxman et al. 1982). The density of Na⁺ channels increases in the CNS from birth to P21, followed by a significant decrease in the adult (Xia and Haddad 1994). The change in the density of Na⁺ channels is accompanied by a proportionate change in “persistent” Na⁺ current (Alzheimer et al. 1993), which is important to the development of anoxic injury in CNS white matter (Stys et al. 1993). Within the optic nerve, Na⁺ channels begin to aggregate in high density at the developing nodes of Ranvier during early myelination, between P11 and P20 (Waxman et al. 1982). That Na⁺ channel density is maximal at P21 is interesting considering the heightened sensitivity of white matter to energy deprivation observed at that age. In addition to changes in Na⁺ channels, neuronal Ca²⁺ currents also increase in magnitude in the postnatal period (Lorenzon and Foehring 1995), and it has been observed recently that Ca²⁺ channels also contribute to the development of anoxic injury in white matter (Fern et al. 1995).
gray matter, suggesting that the small amount of ATP generated by glycolysis, which can proceed in the absence of oxygen, was sufficient to stave off irreversible injury in axons but not in neuron somata or synaptic terminals.

Glucose withdrawal

For several reasons it is likely that removal of bath glucose caused whole nerve glucose levels to fall rapidly, within a few minutes, to nominally zero: 1) the extracellular glucose concentration in the CNS is in near equilibrium with cytoplasmic glucose concentration (Silver and Ereinska 1994). Cytoplasmic glucose will fall quickly, therefore, when bath glucose is removed. 2) The concentration of extracellular glucose within the nerve is undoubtedly lower than bath glucose concentration due to the high rate of brain glucose utilization. Extracellular glucose concentration is about one-third of blood glucose concentration in rat brain (Silver and Ereinska 1994), where its metabolism is phosphorylation rather than transport limited (Furler et al. 1991). 3) And even if the cytoplasmic glucose concentration was as high as 5 mM (under control conditions where bath glucose is 10 mM), this would be consumed within mins in the absence of a continued glucose supply, given a glucose consumption rate of 0.5–1.0 mM per mouse per kilogram brain tissue (Clarke and Sokoloff 1994).

CAP area was maintained for 40–45 min after the withdrawal of glucose, and a 45-min period of perfusion with glucose-free ACSF produced no permanent loss of function. This observation shows that the energy requirements of the optic nerve can be temporally met by energy reserves in the absence of an exogenous energy supply. The major energy reserve of the CNS is glycogen (e.g., Lowry et al. 1964), and there is good evidence to support the idea that astrocytes, the only cells in the CNS with significant stores of glycogen (Brückner and Biesold 1981; Swanson 1992), can pass energy substrate to neurons by releasing lactate to fuel adjacent neurons (e.g., Poitry-Yamate et al. 1995; Tascopoulos and Magistretti 1996). The persistence of the CAP in the absence of glucose and in the presence of oxygen, which is required for lactate metabolism, may be due in large part to the capacity of axons to derive ATP from lactate generated by astrocytes. Other energy reserves, such as amino acids and phospholipids, also will be available to axons, and these smaller reserves are likely to contribute to the maintenance of function in axons after the withdrawal of glucose (Siesjo 1988).

Sixty minutes of glucose withdrawal permanently reduced CAP area to 42.3% of control, and 65 min of glucose withdrawal caused complete and irreversible loss of the CAP. Presumably the energy reserve of the optic nerve is exhausted after ~45 min of glucose withdrawal, an event that is followed by almost simultaneous loss of the CAP and onset of permanent injury. During anoxia, the CAP is lost in a transient fashion within the first few minutes and can be restored with minimal permanent injury if the period of anoxia is brief (e.g., Fig. 2). This difference presumably reflects the different sources of energy available under the two conditions. During anoxia, optic nerves can metabolize anaerobically glucose to lactate yielding a small but continuous supply of energy. During glucose withdrawal, optic nerves can metabolize aerobically any intrinsic energy reserve with a high yield of ATP. When the energy reserve is gone, however, the optic nerve will experience complete failure in energy supply and transient conduction block and permanent loss of function follow in rapid sequence.

We consistently observed that, after the withdrawal of glucose, the later components of the CAP were lost before the early component, and the early component recovered to a greater degree after reperfusion with normal ACSF. This was not observed during anoxia (compare Figs. 1 and 5) or during joint anoxia and glucose withdrawal (not shown). The mechanism underlying this phenomenon is unclear, but presumably involves differences in the ability of different populations of axons within the optic nerve to use available energy reserves and/or to withstand the loss of energy supply. This is the first time that a functional difference, other than conduction velocity, has been reported in a subpopulation of optic nerve axons.

Combined oxygen + glucose withdrawal

The extent of CAP recovery from 60 min of combined glucose + oxygen withdrawal was significantly lower in adult optic nerves than recovery from 60 min of anoxia in the presence of glucose (19.5 ± 8.6%, n = 9 compared with 37.5 ± 11.1%, n = 41; P < 0.0001). The presence of glucose during anoxia, therefore, mitigates the development of injury despite yielding a maximum of only two ATP per glucose via anaerobic metabolism. CAP recovery from 60 min of combined glucose + oxygen withdrawal was also significantly lower than recovery from 60 min of glucose withdrawal (19.5 ± 8.6%, compared with 42.3 ± 10.0%, P < 0.0001). These observation are consistent with the idea that a low level of ATP production, either from anaerobic metabolism of glucose or aerobic metabolism of energy stores, can have a significant impact on the ability of CNS white matter to survive energy deprivation.

Premyelinated optic nerves (P0–P4) exhibited a degree of resistance to combined glucose + oxygen withdrawal not found at later developmental stages. This developmental period corresponds to a time of increased glycogen deposition in the CNS (Kohle and Vannucci 1977). Glycogen is the largest energy reserve in the CNS (e.g., Lowry et al. 1964). Generally, it is thought that glycogen in the CNS is located only in glial cells and not in neurons or axons (Brückner and Biesold 1981; Swanson 1992). Axons in the very young CNS might better withstand glucose withdrawal because they have access to a larger glycogen energy reserve. Increased glycogen in glial cells in the neonatal CNS cannot explain the tolerance of P0–P4 white matter to combined oxygen + glucose withdrawal because lactate or pyruvate, the energy substrates most likely transferred from glia to neurons, cannot be metabolized in the absence of oxygen.

The difference in CAP recovery from 60-min periods of zero-glucose, with or without oxygen, is illustrated for animals of different ages by the shaded area in Fig. 13B. It is apparent that the oxygen-dependent energy reserve is significant in CNS white matter, particularly between P5 and P20 and in the adult (Fig. 13B). The nature of this energy reserve is not yet clear and probably includes the glycogen present in astrocytes, as well as amino acids and phospholipids (Siesjo 1988). Considering that glycogen is by far the
FIG. 13. Summary diagram of dysfunction and injury patterns seen in optic nerves exposed to different forms of energy deprivation. A: typical changes in CAP area recorded from adult optic nerves during and after a 60-min period of glucose withdrawal (−Glucose, +O₂) or combined zero-glucose + anoxia (−Glucose, −O₂). Because the difference in these conditions reflects the ability (in the presence of oxygen), or the inability (in the absence of oxygen), of the optic nerve to metabolize oxygen-dependant stores and because astrocytic glycogen is the largest component of such stores, the difference between these 2 plots may represent the importance of astrocytic glycogen for optic nerve function and survival after the withdrawal of glucose (shaded area). B: diagram showing the difference between the CAP recovery found after 60 min of glucose withdrawal and 60 min of combined glucose + oxygen withdrawal (shown as CAP recovery in A), at different ages. Difference in recovery between these 2 conditions represents the contribution made by oxygen-dependant stores to white matter survival during glucose withdrawal. Such stores contributes significantly to optic nerve survival under conditions of substrate deprivation at all ages after P4–P5, and was most significant between P10 and P20.

largest energy reserve in the CNS (e.g., Lowry et al. 1964) and that glycogen is restricted to astrocytes, these observations provide the first evidence for metabolic support of axons by astrocytes in white matter. Glial support of neurons in other preparations has been well documented (e.g., Poitry-Yamate et al. 1995; Tsacopoulos et al. 1994).

Glycogen may be present transiently in immature axons (Brückner and Biesold 1981), and glycogen within axons, as opposed to glycogen within glia, would be accessible for maintaining the integrity of axons during joint glucose + oxygen withdrawal (glycogen could be metabolized to lactate). It is possible, therefore, that axonal energy stores in the form of glycogen may contribute to the heightened tolerance of perinatal white matter to the conditions that exist during ischemia in the CNS. Alternatively, the maturation and density of ion channels may be an important factor (Alzheimer et al. 1993; Waxman et al. 1989; Xia and Haddad 1994). Considering that white matter is the site of clinically important injuries associated with energy deprivation in the immature CNS, a better understanding of the resistance of neonatal white matter to the breakdown of energy utilization is an important future goal.

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