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Magnetic Resonance Imaging of Brain Edema in the Neonatal Rat: A Comparison of Short and Long Term Hypoxia-Ischemia

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ABSTRACT

Diffusion-weighted and transversal relaxation time (T_2)-weighted magnetic resonance imaging were used to study the relationship between the duration of hypoxia-ischemia [unilateral common carotid artery (CCA) ligation and exposure to 8% oxygen] and the *in vivo* visualization of brain edema in 7-d-old rats. After CCA ligation, 35 animals were divided into five groups according to the length of exposure to 8% oxygen: no exposure (n = 9), 15 min (n = 12), 30 min (n = 5), and 1 h (n = 9) exposure; six animals served as controls. Diffusion weighted images were acquired 2 h after the hypoxic-ischemic insult, sequential T_2 weighted images were recorded for up to 7 d and the outcome was documented by histologic examination at 21 d. The apparent diffusion coefficient of water in the ipsilateral cortex was significantly decreased in all animals recovering from prolonged hypoxic-ischemic insult (30 min and longer), whereas this was the case in only 40% of animals exposed to 15 min of hypoxia. Moreover, T_2 prolongation of brain tissue occurred only in the former group. These results indicate transient and reversible alterations of physiologic water compartmentation for short term hypoxia-ischemia, but irreversible edema formation for long term hypoxia-ischemia. They support the hypothesis that the duration of hypoxia-ischemia determines whether a vasogenic edema and infarction follows the initial cytotoxic edema. (***Pediatr Res* 38: 113–118, 1995**)

Abbreviations

ADC_w, apparent diffusion coefficient of water
CCA, common carotid artery
DWI, diffusion weighted imaging
ECMO, extracorporeal membrane oxygenation
GFAP, glial fibrillary acidic protein
MRI, magnetic resonance imaging
RARE, rapid acquisition with relaxation enhancement
T_2, transversal relaxation time
T2WI, T_2 weighted imaging
T_M, mixing time
T_E, time to echo
T_R, time to repeat

Over the past few years, a large number of animal studies have contributed to a better understanding of the pathogenesis of perinatal hypoxic-ischemic brain damage (1–8) and accompanying cerebral edema (2). This is largely due to the adaptation of the Levine preparation (9)—unilateral CCA ligation with subsequent hypoxia—to 7-d-old neonatal rats (1), whereby unequivocal unilateral brain damage can be produced. This animal model admits to titrate the severity of hypoxic-ischemic lesions by varying the duration of hypoxia (exposure to 8% oxygen). The extent of brain damage to the ipsilateral hemisphere was classified as mild cerebral edema after 1 h of hypoxia, or progressively increasing edema after 3 h of hypoxia (2). In particular, no brain damage was observed after CCA ligation alone (1, 2). This is an important observation, *e.g.* in the context of ECMO for severe respiratory failure in newborn infants, which involves permanent unilateral CCA ligation (10). In this regard, ECMO bears the risk of hypoxic-ischemic encephalopathy resulting from the combination of hypoxia and hypoperfusion during or after the procedure (11, 12).

The noninvasive nature of MRI together with the sensitivity of the contrasting MRI method to the physical environment of water molecules allows the assessment of the nature and extent of brain edema *in vivo* (13, 14). Among the large number of MRI techniques that have been developed to provide optimal contrast for various applications, DWI (15) and T2WI allow differentiation between the cytotoxic, *i.e.* the intracellular

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edema (16, 17), and the vasogenic, *i.e.* the extracellular edema (16, 17), that develop within hours and days, respectively, after a hypoxic-ischemic insult.

The purpose of this study was to combine DWI and T2WI to investigate the threshold duration of hypoxia causing brain edema in conjunction with unilateral CCA ligation. A major objective of this study on the nature and time course of brain edema has addressed the questions of *1*) whether hyperintensity observed in DWI is always indicative of cell damage and subsequent necrosis/gliosis, and *2*) whether DWI might visualize cytotoxic edema associated with a purely ischemic insult.

METHOD

Animal preparation. These studies were approved by the Animal Ethical Committee of the University of Zurich. In our laboratory, a total of 41 7-d-old postnatal Zur:SIV rats (of Spargue-Dawley background) were investigated. Six animals comprised the control group (group A), whereas 35 rats were anesthetized with diethyl ether and were then subjected to unilateral (right) CCA occlusion under a warming lamp. Nine rats were kept under room air (group B), whereas 26 rats were further exposed to 8% oxygen for spontaneous breathing. The duration of hypoxia was 15 min in 12/26 animals (group C), 30 min in 5/26 animals (group D), and 1 h in 9/26 animals (group E). All rats were kept in a thermal box which guaranteed a rectal temperature of 37 ± 0.5°C (8) measured by a copper constantan thermocouple (Marlin, Cleveland, OH). During the experimental MRI procedure, the rats were wrapped to maintain body temperature and were anesthetized with a mixture of oxygen and nitrous oxide (1:3) and 0.75% halothane.

MRI methods. Data were acquired on a Biospec 47/30 4.7 tesla system (Bruker-Spectrospin, Fällanden, Switzerland) using a 20-cm actively shielded gradient insert for gradient field strengths of up to 100 millitesla/m. A 25-mm birdcage coil was tuned to 200 MHz for radiofrequency excitation and reception of ^{1}H NMR signals. T2WI was taken by means of the multislice RARE technique (18). The interecho interval was 22 ms. The RARE factor was 16, and T_R 4000 ms. Spatial resolution was 115 × 230 μm (128 phase encoding steps) and slice thickness 2 mm. As DWI sequence the stimulated echo imaging technique was used (19), placing the pair of diffusion sensitizing gradients symmetrically to the T_M. The sequence parameters were T_E 22 ms, T_M 100 ms, T_R 1800 ms, and a diffusion weighting factor, b, of 1400 s/mm^2 was used (20). To reduce motion artefacts, multiple averaging (n = 8) was performed at reduced spatial resolution in one direction (64 phase encoding steps).

MRI examination protocol. DWI was performed 2 h after the ischemic or hypoxic-ischemic insult; T2WI was done at 3 h, 2 d, and 7 d.

Histologic examination. Randomly selected rats of each group were killed for histopathologic examinations at 21 d. The rats were perfused under deep Rompun/Ketanest anesthesia with 200 mL 4% paraformaldehyde in 0.01 M PBS, pH 7.4. Brains were removed and postfixed in the same fixative for 3 h at 4°C, then dehydrated in ascending alcohols and embedded in paraffin according to standard techniques. Four-micrometer paraffin sections were cut at the levels of the dorsal hippocampus, the dorsolateral striatum, and at the brainstem, including the cerebellum at the level of the facial nucleus. Paraffin sections were stained with hematoxylin-eosin, Luxol (Dupont, Wilmington, DE), and cresyl violet for routine histology. In addition, sections were immunostained either for the astrocytic marker using a polyclonal anti-cow GFAP (21) antibody (DAKO, Hamburg, Germany) or for the neuronal marker synaptophysin using a monoclonal anti-human synaptophysin (22) antibody (Boehringer Mannheim, Germany). Antibody detection was performed using an avidin-biotin-peroxidase complex with diaminobenzidine as substrate.

RESULTS

MRI

A total of 41 animals were investigated. According to the experimental procedure, the neonatal rats were categorized into five groups as shown in Table 1.

Group A. Figure 1 demonstrates an axial T2WI of the forebrain of an age matched control animal, which was subjected neither to carotid ligation nor to hypoxia. Note the high signal intensity of subcortical white matter in the immature brain. DWI of the control animals did not show any abnormalities.

Group B. The imaging results with regard to CCA ligation without hypoxia were uniform: no abnormal changes of signal contrast, in either DWI or in T2WI, were seen in any of the cerebral regions (Fig. 2*A*).

Group C. Animals exposed to 8% oxygen for 15 min showed two different types of MRI patterns. In 40% of the animals a hyperintensity in DWI confined to the ipsilateral cerebral cortex was followed by normal findings in T2WI after 2 d of recovery (Fig. 2*B*). Sixty percent of the animals showed no changes in MRI signal intensity, in either DWI or in T2WI.

Groups D and E. In animals exposed to 8% oxygen for a duration of 30 min and 1 h, respectively, DWI showed a clear

Table 1. *Generation of brain edema of the ipsilateral hemisphere vs the duration of hypoxia*

Group	CCA ligation	Duration of exposure to 8% oxygen	No. of animals with DWI hyperintensity* at 2 h of recovery	No. of animals with T2WI hyperintensity* at 2 d of recovery
A: n = 6	No	No exposure	None	None
B: n = 9	Yes	No exposure	None	None
C: n = 12	Yes	15 min	5 ≈ (40%)	None
D: n = 5	Yes	30 min	5	5
E: n = 9	Yes	1 h	9	9

* Compared to the MR signal intensity of the contralateral hemisphere.

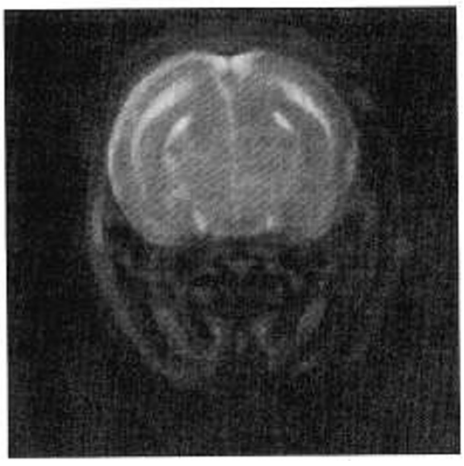

Figure 1. T_2-weighted RARE image of a 7-d-old control rat. The high signal intensity of subcortical white matter is due to the immature brain reflecting a high content of freely diffusible water.

hyperintensity in the ipsilateral hemisphere after 2 h of recovery. These areas involved mainly the cerebral cortex and parts of the basal ganglia. The signal intensity in the two cerebral hemispheres was slightly different in T2WI at this point (hyperintensity of 10% relative to the contralateral side in 6/14 animals), but differed significantly after an interval of 2 d. These hyperintense areas on T2WI corresponded to the hyperintense regions in DWI, but were increased in extent (Fig. 2*C*).

Histology

Group B. The regular neuronal structure and cell density was well preserved in all brain regions examined (Fig. 3*A*). GFAP immunostaining showed a strong, constitutive GFAP expression on astrocytes mainly in the white matter such as the corpus callosum but also in gray matter areas such as the hippocampus. However, only a few astrocytes were GFAP immunoreactive in the neocortex.

Group C. The neuronal damage and astrocytic response were more pronounced than in animals of group B. The histologic examination of a rat brain, in which no DWI hyperintensity was observed, revealed hypoxic damage of a few scattered neurons in the parietal cortex. Neuronal damage was evident mainly as eosinophilic neuronal shrinkage, but not by complete neuronal necrosis, and was accompanied by a weak astrocytic response (Fig. 3*B*). In these animals no true neuronal cell loss was observed and the astrocytic reactions were secondary to these principally reversible neuronal changes. On the other hand, in two animals, which showed a hyperintensity in DWI, true neuronal cell loss was only rarely found. However, moderate neuronal damage occurred in a columnar fashion, *i.e.* extending vertically from the subpial region throughout all cortical layers. In these areas, numerous hypertrophic, occasionally binucleated GFAP-immunoreactive astrocytes were observed. These lesions were most conspicuous at the border zone between the vascular territories of the anterior and of the middle cerebral artery (Fig. 3*C*).

Group E. A large cystic and fully resorbed infarction was found on histology ipsilateral to the side of the CCA ligation. This lesion was demarcated by a pronounced astrocytic glial scar. In the periphery of the lesion, the subpial layer, and even the superficial neuronal layers were preserved in a pseudolaminar fashion. This cystic lesion extended from the striatum to the parietal cortex at the level of the dorsal hippocampus.

DISCUSSION

The findings of the present investigation should be interpreted in relation to the normal age-dependent patterns of changing signal contrast in T2WI of neonatal brains. This is a prerequisite for correct distinction between physiologic and pathologic water content in brain tissue. In control animals (group A), the unmyelinated subcortical white matter is of high signal intensity (Fig. 1) reflecting a high content of freely diffusible water that decreases with progressing myelination. It is worth noting that early detection of raising water content after hypoxia-ischemia may, therefore, be difficult.

The results answer three important questions with reference to the association between severity of hypoxia-ischemia in conjunction with unilateral CCA ligation and *in vivo* visualization of brain edema. *1*) After unilateral ligation alone, neither the DWI contrast nor the T2WI contrast showed any significant difference between the ipsilateral and the contralateral hemisphere. *2*) Cortical hyperintensity observed in DWI after 15 min of hypoxia, which signifies an intracellular (cytotoxic) edema, was transient, lasting less than 12 h. It was neither followed by a vasogenic edema nor by frank infarction. This suggests that the depression of cell metabolism had not yet reached "the point of no return." *3*) A duration of hypoxia of 30 min to 1 h resulted in an unequivocal cytotoxic edema, followed by a more extensive vasogenic edema after approximately 12–24 h, which heralded cerebral infarction as confirmed by histology.

The classical method of determining the content and volume of hemispheric tissue water includes bisectioning of the forebrain, desiccation, and weighing of the specimen. The water content is then calculated as a percentage of the hemispheric weight. Inasmuch as this method maps the integrity of water molecules, only a change in total bulk water, *i.e.* a transfer of water from blood to tissue, would be apparent. By means of this method, an essentially linear increase in tissue volume during the course of hypoxia-ischemia has been demonstrated (2). However, the understanding of the formation of brain edema with regard to the duration of hypoxia-ischemia requires discrimination between a shift of water from the extracellular to the intracellular compartment and a net increase of interstitial fluid. The first mechanism appears to be responsible for the primary cytotoxic brain swelling during hypoxia-ischemia due to an increase of intracellular idiogenic osmoles and to a failure of the energy-dependent NA^+/K^+ pumps causing transmembranous water shifts. Changes of motor properties of the cellular matrix can be visualized by the ipsilateral hyperintensity in DWI as the data from groups C, D, and E imply (Fig. 2, *B* and *C*). Although the assumption of freely exchangeable water between intracellular and extracellular compartments made for diffusion measurements in healthy brains may not fully apply to hypoxic-ischemic tissue, the decline of the so-called ADC_w (23) implied by the increase of signal intensity

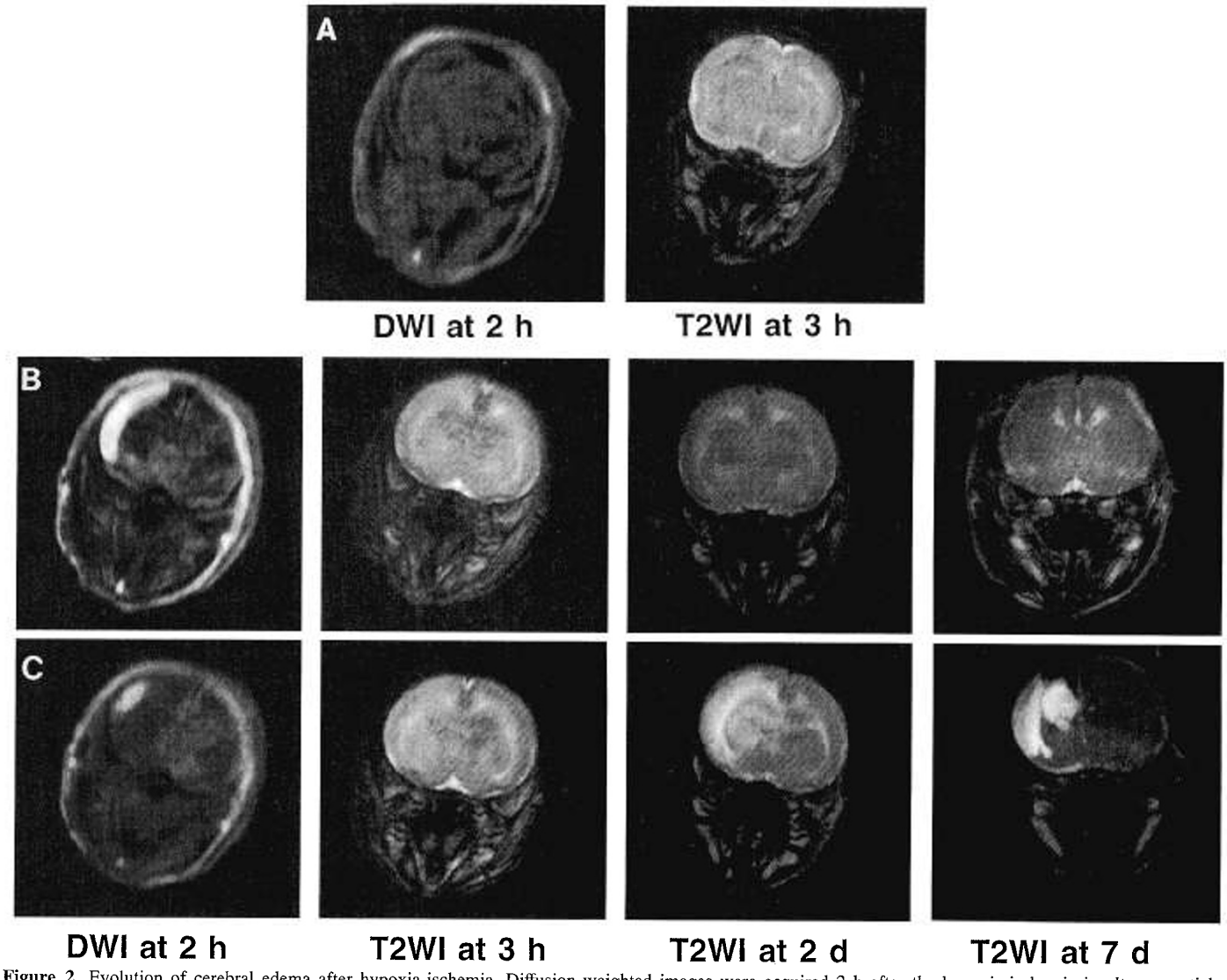

Figure 2. Evolution of cerebral edema after hypoxia-ischemia. Diffusion weighted images were acquired 2 h after the hypoxic-ischemic insult, sequential T_2-weighted images were recorded for up to 7 d. Imaging results for CCA ligation without hypoxia (*A*), 15 min of hypoxia (*B*), and 1 h of hypoxia (*C*) are shown. No differences between the two hemispheres were seen in *A*. Cortical hyperintensity observed in DWI was transient and was not followed by an ipsilateral hyperintensity in T2WI in animals exposed to 15 min of hypoxia (*B*), whereas persistent changes became visible in the ipsilateral cortex of animals recovering from prolonged hypoxia (*C*). Note the high signal intensity of immobile protons of the s.c. fat layer in DWI.

probably reflects a weighted sum of the two compartments as the experimental diffusion sensitizing time (T_M = 100 ms) was long enough for water exchange to take place. Moreover, the intracellular ADC_w is about an order of magnitude smaller than the extracellular ADC_w (24, 25). A narrowing of the extracellular space (26–28) due to the swelling of the cells may, therefore, cause at least part of the ADC_w reduction. Because the cytotoxic edema can be visualized during the first 2–6 h, it implies that the primary shift of water from extracellular to intracellular space is not yet entirely compensated by a subsequent transfer of water from blood to hypoxic-ischemic tissue after blood-brain barrier breakdown. In contrast, the accumulation of interstitial fluid as in vasogenic edema and subsequent tissue necrosis and gliosis may be visible by high signal intensity of T2WI. Such T_2 prolongations reflect a dramatic increase of freely diffusible tissue water (Fig. 2*C*). They were only detected in the animals exposed to 30 min and 1 h of hypoxia (groups D and E). As the vasogenic edema can spread out along fiber tracks preferably along association fibers, the extent of the vasogenic edema is not confined to the extent of the primary cytotoxic edema.

Based on the observation that, after 15 min of hypoxia, cellular swelling was not followed by T_2 prolongation of brain tissue nor by any increase in water content, we are left with the question of what metabolic effect underlies a short term hypoxia-ischemia. In making a hypothesis within this context, the following two findings are weighty. First, the normal pattern of T2WI contrast observed 2 d after recovery was entirely consistent with that seen in animals with CCA ligation alone and, notably, in animals of the control group. Second, the occurrence of isolated selective neuronal necrosis indicated that the hypoxic-ischemic brain tissue was still a functionally and structurally intact zone. Thus, it is reasonable to assume that the reestablishment of physiologic water compartmentation after short term hypoxia-ischemia occurs as a consequence of a recovered energy metabolism. Therefore, the dependence of

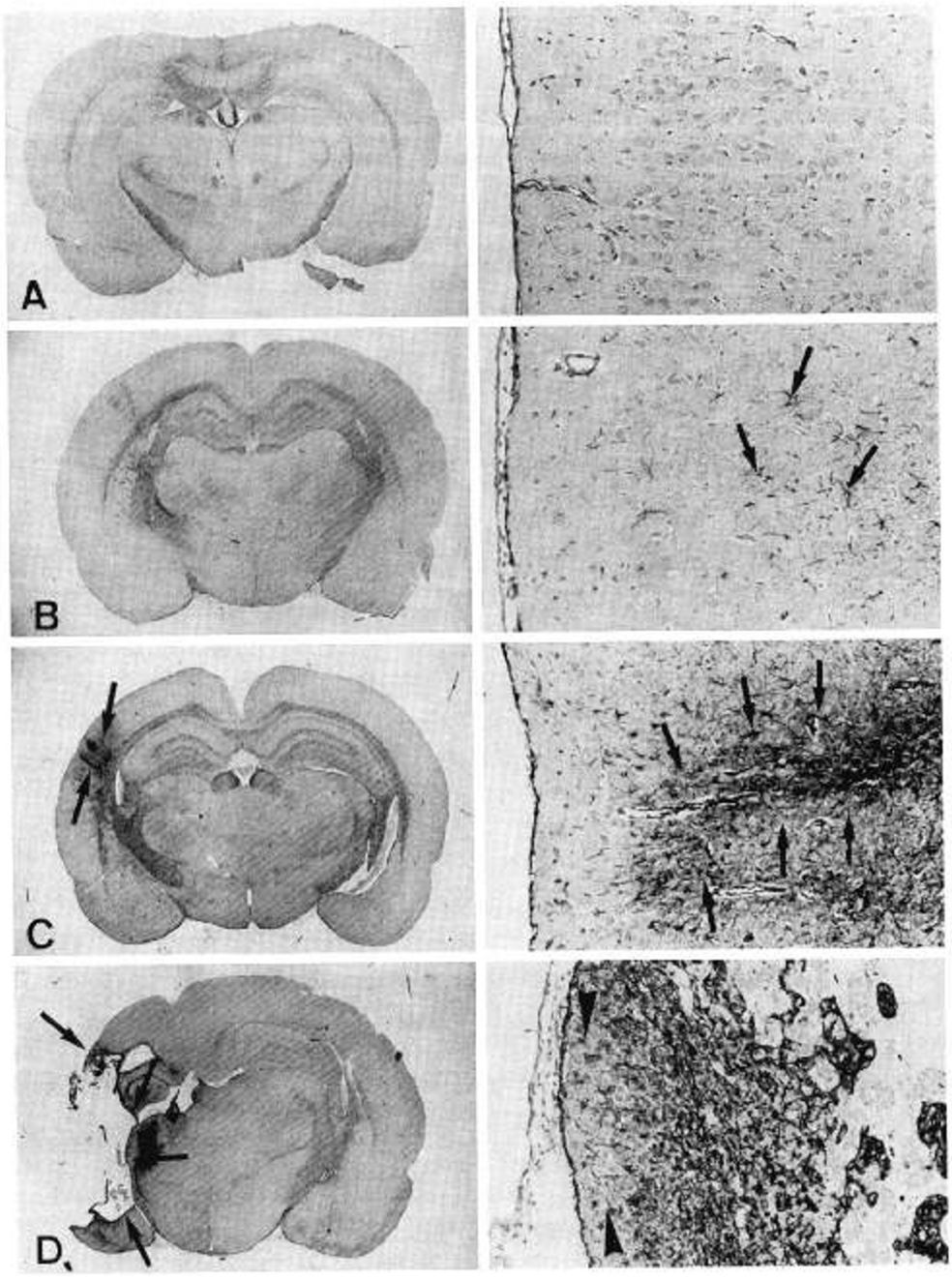

Figure 3. GFAP immunocytochemistry for reactive astrocytes. The brain sections were cut at the level of the dorsal hippocampus at 21 d (*left column*, overview; *right column*, higher magnification of parietal cortex). Section in *A* demonstrates that CCA ligation without hypoxia produces no neuronal damage. Section in *B* demonstrates only a few scattered GFAP-immunoreactive astrocytes (*arrows*) in the parietal cortex after 15 min of hypoxia-ischemia. In this case, no DWI hyperintensity was observed. When DWI hyperintensity was observed, numerous GFAP-immunoreactive astrocytes demarcated neuronal cortical damage in a columnar fashion (*arrows* in *C*). Section in *D* demonstrates the outcome of hyopxia of 1 h duration. Almost one entire cortical hemisphere with the underlying subcortical structures has undergone necrosis and is replaced by a huge cystic infarction (*arrows*). Higher magnification (*right column*) shows that the subpial layer is preserved in a pseudolaminar fashion (*arrowhead*). Original magnifications: left column, ×20; right column, ×180.

brain water content on duration of hypoxia might, in turn, be biphasic rather than linear. Phase A of the hypothetical curve in Figure 4 represents a shift of water between the extracellular and intracellular compartment, *i.e.* cytotoxic edema, with only an insignificant increase of total brain water, whereas phase B describes an essentially linear increase in the extent of brain edema. We call the curvilinear zone at around 15 min of hypoxia the hypoxic-ischemic threshold. Support for this explanation is also derived from investigations in other animal models, such as the rat stroke model of middle cerebral artery occlusion (28) and the four-vessel occlusion model (29). It has to be pointed out that, for the various animal models and whether immature or adult animals (4), reduction in cerebral blood flow may differ and, thus, brain edema evolution may occur time-shifted (30).

The following two observations in rats recovering from long term hypoxia-ischemia are in agreement with the hypothesis of a biphasic development of edema. First, the extent and inten-

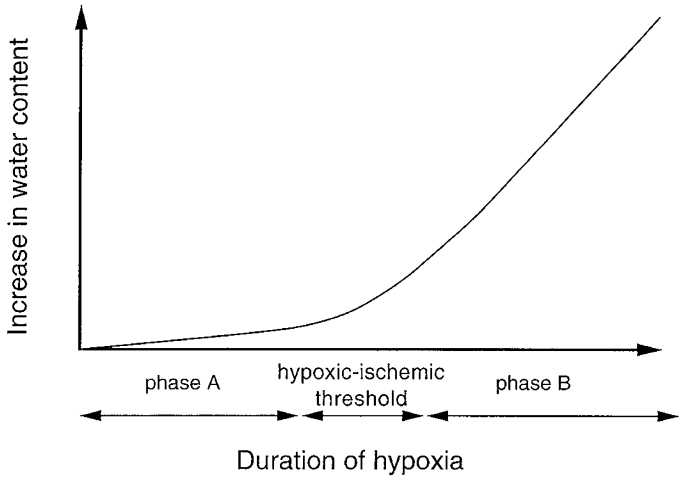

Figure 4. Schematic representation of the dependence of brain water content on duration of hypoxia-ischemia. The curvilinear zone is called the hypoxic-ischemic threshold.

sity of the hyperintense area in DWI are smaller compared with that of short term hypoxia-ischemia. Second, in a certain percentage of T2WI taken within the same time frame, some early hyperintensity from vasogenic brain edema are visible. Both effects may be due to a more severe blood-brain barrier breakdown caused by the longer duration of hypoxia-ischemia. The subsequent bulk water uptake into the extracellular compartment (phase B in Fig. 4) counteracts the abnormal uptake of water by injured brain cells in a more pronounced manner, and thus leads to a weakening of the underlying DWI effect.

It is apparent from this study that a primary disturbance of physiologic water compartmentation leading to a cytotoxic edema is reversible for short term hypoxia-ischemia, whereas long term hypoxia-ischemia generates bulk water shifts resulting in vasogenic edema and subsequent necrosis and gliosis. Therefore, we conclude that a tolerance interval for the duration of hypoxia must exist after which a disturbed brain metabolism and its cellular consequences, *e.g.* cell membrane integrity, are largely reversible. It follows that DWI hyperintensity *per se* does not implicate a permanent brain lesion.

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