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Enlarged extracellular space of aquaporin-4-deficient mice does not enhance diffusion of Alexa Fluor 488 or dextran polymers

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Abstract

Aquaporin-4 (AQP4) water channels expressed on glia have been implicated in maintaining the volume of extracellular space (ECS). A previous diffusion study employing small cation tetramethylammonium and real-time iontophoretic (RTI) method demonstrated an increase of about 25% in the ECS volume fraction (α) in the neocortex of AQP4^{-/-} mice compared to AQP4^{+/+} mice but no change in the hindrance imposed to diffusing molecules (tortuosity λ). In contrast, other diffusion studies employing large molecules (dextran polymers) and fluorescence recovery after photobleaching (FRAP) method measured a decrease of about 10–20% in λ in the neocortex of AQP4^{-/-} mice. These conflicting findings on λ would imply that large molecules diffuse more readily in the enlarged ECS of AQP4^{-/-} mice than in wild type but small molecules do not. To test this hypothesis, we used integrative optical imaging (IOI) to measure tortuosity with a small Alexa Fluor 488 (MW 547, λ_{AF}) and two large dextran polymers (MW 3,000, λ_{dex3} and MW 75,000, λ_{dex75}) in the *in vitro* neocortex of AQP4^{+/+} and AQP4^{-/-} mice. We found that $\lambda_{AF} = 1.59$, $\lambda_{dex3} = 1.76$ and $\lambda_{dex75} = 2.30$ obtained in AQP4^{-/-} mice were not significantly different from $\lambda_{AF} = 1.61$, $\lambda_{dex3} = 1.76$, and $\lambda_{dex75} = 2.33$ in AQP4^{+/+} mice. These IOI results demonstrate that λ measured with small and large molecules each remain unchanged in the enlarged ECS of AQP4^{-/-} mice compared to values in AQP4^{+/+} mice. Further analysis suggests that the FRAP method yields diffusion parameters not directly comparable with those obtained by IOI or RTI methods. Our findings have implications for the role of glial AQP4 in maintaining the ECS structure.

Keywords

glia; tortuosity; IOI; FRAP; epilepsy

Glial aquaporin-4 (AQP4) is a major water channel in brain. AQP4 channels expressed on glial processes at brain-fluid interface (e.g. astrocytic perivascular and subpial endfeet) have been implicated in cerebral water balance (Frigeri et al., 1995, Nielsen et al., 1997, Manley et al.,

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2000; Amiry-Moghaddam et al. 2003; Amiry-Moghaddam and Ottersen 2003; Manley et al., 2004). AQP4 channels are also expressed at nonend-feet glial membranes within the brain neuropil; these channels are proposed to be involved in the activity-dependent water transport between ECS and glia (Nagelhus et al., 1998; Amiry-Moghaddam et al., 2004a; Amiry-Moghaddam et al., 2004b; Nagelhus et al., 2004). A recent diffusion study (Yao et al., 2008) employing the real-time iontophoretic (RTI) method (Nicholson and Phillips, 1981) addressed the question whether AQP4 channels are functionally significant for maintaining the volume of brain extracellular space (ECS) under resting physiological conditions. It found that the ECS volume fraction ($\alpha = V_{\text{ECS}}/V_{\text{tissue}}$, where V represents volume) was increased by about 25% in the neocortex of AQP4^{-/-} mice compared to AQP4^{+/+} mice. Yao et al. (2008) argued that the increased ECS volume fraction may account for the elevated seizure threshold observed in the AQP4^{-/-} mice (Binder et al., 2004a; Binder et al., 2006). An increased ECS volume fraction of AQP4^{-/-} mice (Yao et al., 2008) indicates that deletion of the glial AQP4 channel alters the structure of brain ECS. However, there have been conflicting reports as to whether tortuosity, the other major structural parameter of ECS, changes in AQP4^{-/-} mice.

Tortuosity ($\lambda = (D/D^*)^{0.5}$, where D is the free diffusion coefficient and D^* is the effective diffusion coefficient in the tissue) quantifies the hindrance imposed on the diffusing molecules by the ECS (Nicholson, 2001; Hrabětová et al., 2004). Yao et al. (2008) showed that λ obtained with small tetramethylammonium cation (TMA, MW 74) remained unchanged in the neocortex of AQP4^{-/-} mice, both *in vivo* and *in vitro*. In contrast, a decrease of about 10–20% was detected when λ was quantified by the FRAP method using large molecules (fluorophore-labeled dextran polymers, MW 4,000, 70,000, and 500,000) in the neocortex of AQP4^{-/-} mice *in vivo* (Binder et al., 2004b; Papadopoulos and Verkman, 2005; Zador et al., 2008).

Taken together, these diffusion studies of AQP4^{-/-} genotype ECS lead to the conclusion that there is a significant change in the hindrance imposed on large molecules but not small ones. This is surprising because previous work demonstrated that when the ECS is altered, the accompanying changes in λ measured with both small and large molecules were qualitatively similar. For example, both λ_{TMA} and λ_{dex3} increased when the ECS was reduced by hypotonic or ischemic insult (Tao 1999; Kume-Kick et al., 2002; Hrabětová et al., 2003). Here we tested the hypothesis that large molecules diffuse more readily in the ECS of AQP4^{-/-} mice than in wild type but small molecules do not. To this end, we used a method of integrative optical imaging (IOI; Nicholson and Tao, 1993) to measure λ with both small fluorophores (Alexa Fluor 488; AF, MW 547) and large fluorophore-labeled dextran polymers (dex3, MW 3,000; dex75, MW 75,000) in the *in vitro* neocortex of AQP4^{+/+} and AQP4^{-/-} mice.

Experimental procedures

Neocortical slice preparation

Experiments were performed at New York University School of Medicine in accordance with the NIH guidelines and local IACUC regulations. The AQP4^{-/-} mice generated in a CD1 genetic background (Ma et al., 1997), and AQP4^{+/+} mice matched in age and body weight, were obtained from Professor Geoffrey T. Manley, University of California, San Francisco. A total of 6 AQP4^{+/+} and 5 AQP4^{-/-} 4–5 month-old male mice weighting 35–45 g were used. The animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and decapitated with a guillotine. The brain was extracted from the skull and chilled with ice-cold artificial CSF (ACSF). The composition of ACSF was (in mM): NaCl 124, KCl 5, NaHCO₃ 26, NaH₂PO₄ 1.25, D-glucose 10, MgCl₂ 1.3, CaCl₂ 1.5. The ACSF was gassed with a mixture of 95% O₂ and 5% CO₂ to buffer the pH at 7.4. The osmolality of ACSF, 295–305 mosmol/kg, was determined with a freezing point–depression osmometer (Osmette A #5002; Precision Systems Inc., Natick, MA, U.S.A.). Coronal brain slices were cut 400 μm thick using a vibrating-blade microtome (VT 1000S; Leica Instrument GmbH, Nußloch, Germany). After dissection, the

slices were incubated in the ACSF at room temperature for at least one hour before the measurement to allow for their recovery. A single slice was then transferred to a submersion recording chamber (model RC-27L; Warner Instruments, Hamden, CT, U.S.A.) and superfused with ACSF at a flow rate of 2.0 mL/min. The temperature was maintained at 34 ± 1 °C by a temperature controller (model TC-344B; Warner Instruments, Hamden, CT, U.S.A.). All measurements were made in the primary somatosensory neocortex.

Molecules

Three molecules were used in this study: fluorophore AF (catalog #A10436, Invitrogen, Carlsbad, CA, U.S.A.), Texas Red-labeled dex3 (catalog #D-3329, Invitrogen), and FITC-labeled dex75 (catalog #FD-70S, Sigma-Aldrich, St. Louis, MO, U.S.A.). These molecules were dissolved in a solution of 150 mM NaCl to a final concentration of 1 mM for AF and dex3, and 0.1 mM for dex75. Diffusion of each molecule was measured in brain slices prepared from at least three animals of each genotype.

Integrative optical imaging (IOI) method

The experimental system for the IOI method and the related theory have been described in detail previously (Nicholson and Tao, 1993; Tao and Nicholson, 1995). Briefly, a glass micropipette pulled from thin-wall glass tubing (catalog #6170, A-M System, Carlsborg, WA, U.S.A.) was loaded with fluorescent molecules. The tip, 2–4 μm in diameter, was positioned 200 μm below the surface of the brain slice or 1000 μm into agarose gel (0.3% in 150 mM NaCl; NuSieve GTG Agarose, FMC BioProducts, Rockland, ME, U.S.A.). The fluorescent molecules were released by a brief pressure pulse (10–200 ms, 10–20 psi) of compressed nitrogen. A time-series of the 2-dimensional (2-d) projections of the diffusion cloud were captured by a CCD camera (model CH350, Photometrics, Tucson, AZ, U.S.A.) attached to an Olympus BX61WI compound microscope (Olympus America, Melville, NY, U.S.A.) equipped with a water immersion objective (Olympus UM PlanFl 10 $\times$, NA 0.3). Image acquisition and data analysis were performed using MATLAB-based (The MathWorks, Natick, MA, U.S.A.) programs (Hrabětová and Nicholson, 2007). From each image in the series, six intensity profiles intersecting at the image center at an angle of 0°, 30°, 60°, 90°, 120°, and 150° from the horizontal axis were extracted and fitted with Gaussian curves to obtain $\gamma^2/4$ values, where $\gamma^2 = 4D^*(t_i + t_0)$ and t_i is the time at which the image was taken and t_0 is a time offset that compensates for the finite size of the injection; see Nicholson and Tao (1993) and Prokopová-Kubinová et al. (2001) for details. The lower 10% of intensity profiles were excluded from fitting. A linear regression was then applied to these six series of $\gamma^2/4$ against t_i to estimate D^* (cm^2s^{-1}) in brain slices and D (cm^2s^{-1}) in agarose gel. The minimum and maximum values of diffusion coefficient were excluded to increase the robustness of an estimate, and the four remaining values were averaged. The tortuosity λ was then calculated for each image series obtained in brain slices.

Statistics

SigmaStat 3.5 (Systat Software, San Jose, CA, U.S.A.) was used for statistical analysis of data obtained in brain slices. The Two Way ANOVA with interaction tested for the effect of two factors alone (*genotype* and *molecule*) or in combination on λ . Posthoc all pairwise multiple comparison procedures with Holm-Sidak method tested for the differences between individual groups. Values $P < 0.05$ were considered significant.

Results

Diffusion of AF

Figure 1 shows the results obtained with fluorophore AF in the neocortical slices from AQP4^{+/+} and AQP4^{-/-} mice. Images from a representative time series recorded in AQP4^{+/+} mice and the corresponding intensity profiles are shown in Fig. 1a,b. The $\gamma^2/4$ versus time curve fitted with a linear regression line used to determine D^* are plotted and compared with a representative result from AQP4^{-/-} mice (Fig. 1c). The linear regression lines for AQP4^{+/+} and AQP4^{-/-} mice are parallel with each other which indicates that a similar value for D^* was measured in two genotypes with this molecule. The free diffusion coefficient of AF obtained from measurements in agarose gel (Table 1) was used to calculate λ . The averaged values of λ from all experiments in AQP4^{+/+} or AQP4^{-/-} mice are plotted in Fig. 1d, and presented in Table 1.

Diffusion of dextran polymers

Representative $\gamma^2/4$ versus time curves obtained with dex3 and dex75 are plotted for both genotypes in Figure 2a. The linear regression lines fitted to $\gamma^2/4$ versus time curves are parallel in AQP4^{+/+} and AQP4^{-/-} mice for dex3 and also for dex75 indicating that D^* is again similar for each molecule in AQP4^{+/+} and AQP4^{-/-} mice. The free diffusion coefficients of dex3 and dex75 used to calculate λ are reported in Table 1. The averaged values of λ obtained in all experiments in AQP4^{+/+} and AQP4^{-/-} mice with both dextran polymers are plotted in Fig. 2b, and presented in Table 1.

Statistical analysis

All tortuosities obtained in the neocortex of AQP4^{+/+} and AQP4^{-/-} mice with AF, dex3, and dex75 are shown in Fig. 3. Mean values of λ did not differ between the *genotypes* ($P = 0.331$), or when the interaction of two factors was considered ($P = 0.705$). However, there was a statistically significant difference in mean values of λ between *molecules* ($P < 0.001$). The pairwise multiple comparisons showed that $\lambda_{AF} < \lambda_{dex3} < \lambda_{dex75}$ ($P < 0.05$).

In summary, each value of λ_{AF} , λ_{dex3} and λ_{dex75} in the neocortex of AQP4^{-/-} mice were not significantly different from its counterpart measured in AQP4^{+/+} mice. In both genotypes, $\lambda_{AF} < \lambda_{dex3} < \lambda_{dex75}$.

Discussion

We tested the hypothesis that small molecules are hindered equally in the enlarged ECS of AQP4^{-/-} mice and in the normal ECS AQP4^{+/+} mice, whereas large molecules are hindered less in AQP4^{-/-} than in AQP4^{+/+} mice. We employed the same technique to measure the diffusion of a small fluorophore (AF) and two large dextran polymers (dex3, dex75) in the *in vitro* neocortex of AQP4^{+/+} and AQP4^{-/-} mice and found that tortuosities did not depend on the genotype for any of these molecules. We also found that λ increased with size of molecule in both genotypes as would be expected from previous studies in the rat brain.

Tortuosity measured with AF and dextran polymers in AQP4^{-/-} mice

Diffusion measurement using the IOI method with AF confirmed that the hindrance for small molecules is unchanged in the increased ECS of AQP4^{-/-} mice (Yao et al., 2008). Although AF is slightly larger and has opposite net charge than the cation TMA used previously (Yao et al., 2008), λ_{AF} is in a very good agreement with $\lambda_{TMA} = 1.61$ in AQP4^{+/+} mice *in vivo* and *in vitro* and $\lambda_{TMA} = 1.62 - 1.64$ in AQP4^{-/-} mice *in vivo* and *in vitro*. The AF result in mice of both genotypes also agrees well with several *in vivo* RTI studies employing TMA in the neocortex of mice with different genetic backgrounds. These RTI studies reported λ_{TMA} equal

to 1.67 in 3–6 months old mice (Anděrová et al., 2001), 1.47–1.50 in 6–8 months old mice of both genders (Syková et al., 2005a), and 1.52–1.55 in 5–9 months old female mice (Syková et al., 2005b).

Diffusion measurements with dex3 and dex75 demonstrated that the hindrance for large molecules in the increased ECS of AQP4^{-/-} mice is also unchanged when compared to AQP4^{+/+} mice. This result disagrees with several FRAP diffusion studies (Binder et al., 2004b; Papadopoulos and Verkman, 2005; Zador et al., 2008) which estimated a decrease of about 10–20% in λ measured with dextran polymers (MW 4,000, 70,000, 500,000) in the *in vivo* somatosensory neocortex of AQP4^{-/-} mice compared to wild type. We employed simulations (Hrabětová and Nicholson, 2007) to illustrate the consequences of *unchanged* versus *decreased* tortuosity for diffusion of molecules released in the enlarged ECS of AQP4^{-/-} mice (Fig. 4). When λ is *decreased*, the diffusion curve peaks earlier than when λ is *unchanged* demonstrating a faster spread of molecules (Fig. 4). Given the role of non-synaptic mechanisms in the initiation of epileptic activity (Schweitzer et al., 1992), enhanced extracellular diffusion would likely facilitate synchronization of firing needed to form an epileptic focus, and therefore appears to be less consistent with an elevated seizure threshold in AQP4^{-/-} mice (Binder et al., 2004a; Binder et al., 2006).

Diffusion parameters measured with IOI and FRAP methods

There are several possible explanations for discrepancy between IOI and FRAP diffusion studies including the differences in diffusing molecules, brain preparations or methods used. The first explanation, based on differences in the diffusing molecules, is unlikely. The dextran polymers used in FRAP studies and dex75 used here were obtained from the same commercial source.

The second explanation, based on differences in the brain preparations, also seems unlikely. In previous RTI study (Yao et al., 2008), a very good agreement between the ECS parameters obtained *in vitro* and *in vivo* was demonstrated in both the AQP4^{+/+} mice ($\lambda_{\text{TMA}} = 1.61$ and $\alpha = 0.19$ *in vitro*; $\lambda_{\text{TMA}} = 1.61$ and $\alpha = 0.18$ *in vivo*) and the AQP4^{-/-} mice ($\lambda_{\text{TMA}} = 1.64$ and $\alpha = 0.23$ *in vitro*; $\lambda_{\text{TMA}} = 1.62$ and $\alpha = 0.23$ *in vitro*).

The third explanation, based on the method of measurement, may account for the discrepancies between our results and the FRAP diffusion studies (Binder et al., 2004b; Papadopoulos and Verkman, 2005; Zador et al., 2008). The recording intervals (and therefore the tissue volumes explored by the diffusing molecules) are significantly shorter in the FRAP method compared to the IOI (or RTI method) and consequently the FRAP and IOI methods thus may report different diffusion parameters. For example, the diffusion of dex75 in AQP4^{+/+} was recorded for about 10 minutes with the IOI method (Fig. 2a) giving $\lambda_{\text{dex75}} = 2.33$ while it took approximately 300 ms to obtain the $t_{1/2}$ value employed in the FRAP methods to determine $\lambda_{\text{dex70}} \approx 1.77$ (see Fig. 5 in Binder et al., 2004b). To illustrate how much the molecules would spread within 300 ms, we estimated that the root mean square displacement, $(6D^*t)^{1/2}$, would be only about 3 μm assuming $D^* = D^*_{\text{dex75}}$ (D^*_{dex70} not reported in Binder et al., 2004b). Considering that the diameters of cellular processes and bodies in the somatosensory neocortex are in the range from sub-micrometer to tens of micrometers, the FRAP methods likely represent the local tissue property while the IOI method, by averaging from a sufficiently large tissue volume, reports the tortuosity according to the concept of volume averaging (Nicholson and Phillips, 1981). In the FRAP time domain, D^* would be expected to be proportional to the squared distance between cells and inversely proportional to the diffusion time (Callaghan 1991, Eq. 13 in Hrabě et al., 2004), and λ would thus decrease in proportion to increased α . Indeed, a 10–20% decrease in λ was reported with the FRAP method in AQP4^{-/-} where we now know that the ECS is enlarged by about 25% compared to the wild type (Yao et al.

2008). We note that in a later FRAP study, where $t_{1/2}$ value for dextran MW 70,000 was 10 times longer, λ value of about 2.07 was reported in AQP4^{+/+} mice (Zador et al., 2008).

We conclude that the direct comparison of diffusion parameters obtained with the IOI and FRAP methods should be done with caution, particularly for large molecules that diffuse slowly. It is likely that the FRAP methods report locally restricted diffusion, which may be relevant for quantification of diffusion within ECS subdomains with the defined cytoarchitecture or within narrow layers of highly laminated structures such as retina.

Size-dependent ECS diffusion

This is the first study employing the IOI method and a set of molecules of different sizes in the mouse brain. Our finding that λ increased with the molecular size in a wild type mice is consistent with previous measurements with IOI method in rat somatosensory neocortex. These IOI studies reported 1.74–2.01 for dex3 and 2.25 for dex70 in slices (Nicholson and Tao, 1993; Tao and Nicholson 1996, Hrabětová et al., 2003; Thorne et al., 2004, Hrabětová, 2005), and 2.04 for dex3 and 2.76 for dex70 in *in vivo* preparation (Thorne and Nicholson, 2006).

The size-dependence of λ is preserved in the increased ECS or AQP4^{-/-} mice. The implications of this finding are discussed below.

ESC structure in AQP4^{-/-} mice

Yao et al. (2008) as well as this study demonstrate that deletion of the AQP4 channel alters the structure of brain ECS by increasing its volume, without a change in tortuosity. Intuitively, one may expect that a change in α is accompanied by a change in λ . However, making predictions for λ based on α values alone has proven problematic because these aggregate parameters are generally independent (Lehmenkühler et al., 1993; Syková et al., 1996; Kume-Kick et al., 2002, Hrabě et al., 2004; Hrabětová 2005).

Our results on λ measured with dextran polymers in the enlarged ECS of AQP4^{-/-} mice may be compared with an earlier IOI study (Tao, 1999), which quantified λ of the ECS enlarged by osmotic stress. Tao (1999) reported a 6% decrease of λ obtained with dex3 in rat neocortical slices where a 20% increase in α was caused by the superfusion with hypertonic ACSF (Kume-Kick et al., 2002). An even larger decrease in λ , by 15%, was measured for dex75 (F. Xiao, and S. Hrabětová, unpublished observations). However, in the AQP4^{-/-} mice where α increased by about 25%, λ measured with dex3 and dex75 was unchanged. These results not only confirm the independence of α and λ parameters but also demonstrate that hypertonic stress and AQP4^{-/-} deletion modify the ECS structure in a fundamentally different way. Indeed, hypertonic stress is likely to affect both glia and neurons while AQP4 deletion is glia-specific. In addition, the persistent ECS enlargement in the AQP4^{-/-} mice resulting from genetic manipulation is likely more complicated than an acute ECS enlargement induced by hypertonic stress because the AQP4 water channels not only facilitate water movement across the cell membrane but may also play a role in cell adhesion (Hiroaki et al., 2006; McCoy and Sontheimer, 2007; but see Zhang and Verkman 2008).

The important question arising from these diffusion studies is how the ECS becomes enlarged in the AQP4^{-/-} mice. Do the interstitial gaps get wider in a homogeneous manner or is the geometry of the ECS altered in a heterogeneous way? We can speculate that the latter explanation is more likely given that AQP4 channels are expressed on glia but not neurons. A selective decrease of glia volume may lead to a heterogeneous change in the interstitial gaps; this conjecture indicates that the mechanism of α increase needs to be elucidated to fully understand the unexpected effect of AQP4 deletion on ECS structure.

In conclusion, our results demonstrate that diffusion behavior of small and large molecules, as well as the size-dependency of λ , are preserved in the increased ECS of AQP4^{-/-} mice. The findings have implications for the role of glial AQP4 channels in maintaining the ECS structure.

Abbreviations

D	free diffusion coefficient
D*	effective diffusion coefficient
IOI	integrative optical imaging
ECS	extracellular space
RTI method	real-time iontophoretic method
FRAP	fluorescence recovery after photobleaching
MW	molecular weight
AQP4	aquaporin-4
TMA	tetramethylammonium
AF	Alexa Fluor 488
dex3	dextran (MW 3,000)
dex75	dextran (MW 75,000)
ACSF	artificial cerebrospinal fluid

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Figure 1.

Diffusion of AF in *in vitro* somatosensory neocortex of AQP4^{+/+} and AQP4^{-/-} mice. (a) Images from AF time sequence obtained in AQP4^{+/+} mouse. White dashed line in the first image taken after the dye release (labeled as $t = 0$ s) represents the horizontal axis used to extract intensity profiles. The scale bar is 100 μm . (b) Intensity profiles (red) fitted with theoretical Gaussian curves (black). (c) A time series of $\gamma^2/4$ values with a linear regression line recorded in AQP4^{+/+} (grey circles) and AQP4^{-/-} (white circles) mice. D^* (34°C) was $20.25 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in AQP4^{+/+} mice and $20.24 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in AQP4^{-/-} mice. The λ_{AF} was 1.60 in both genotypes. (d) The tortuosities (mean and SD) obtained with AF from all experiments.

Figure 2.

Diffusion of dextran polymers in *in vitro* somatosensory neocortex of AQP4^{+/+} and AQP4^{-/-} mice. (a) A time series of $\gamma^2/4$ values recorded with dex3 and dex75 in AQP4^{+/+} (grey circles) and AQP4^{-/-} (white circles) mice. For dex3, D^* (34°C) was $7.45 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in AQP4^{+/+} mice and $7.46 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in AQP4^{-/-} mice. The λ_{dex3} was 1.73 in both genotypes. For dex75, D^* (34°C) was $0.81 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in AQP4^{+/+} mice and $0.85 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in AQP4^{-/-} mice. The λ_{dex75} was 2.42 in AQP4^{+/+} mice and 2.36 in AQP4^{-/-} mice. (b) The tortuosities (mean and SD) obtained with dex3 and dex75 from all experiments.

Figure 3.

Quantile plot of λ values measured with AF and dextran polymers. The values of λ_{AF} , λ_{dex3} and λ_{dex75} obtained in AQP4^{+/+} (black symbols) and AQP4^{-/-} (white symbols) mice sorted in ascending order were plotted as a fraction of the total number of measurements normalized to 1.

Figure 4.

Simulation of extracellular diffusion in AQP4^{+/+} and AQP4^{-/-} mice. Molecules (dex75) were released from a point source (*black circle*; pulse duration 10 ms) into the ECS where α is 0.19 (AQP4^{+/+}) or 0.23 (AQP4^{-/-}) as measured in the mice neocortical slices (Yao et al., 2008). In AQP4^{+/+} genotype, λ obtained in this study (2.33) was used. In AQP4^{-/-} genotype, either *unchanged* λ (2.30) or λ *decreased* by 15% (10–20% was reported; Binder et al., 2004b; Papadopoulos and Verkman, 2005; Zador et al., 2008) was used. The concentration profiles obtained at the distance 30 μ m from the release site are shown. In AQP4^{-/-} genotype (*dashed* and *dotted* lines), the amplitude of diffusion curves is smaller than in AQP4^{+/+} genotype (*solid* line) reflecting larger dilution of molecules released in enlarged ECS (discussed by Yao et al., 2008). In AQP4^{-/-} genotype with *decreased* λ (*dashed* line), the diffusion curve peaks earlier than when λ is *unchanged* (*dotted* line) demonstrating a faster spread of molecules. We note that the result would hold qualitatively also for smaller molecules and ions.

Table 1

Diffusion of AF and dextran polymers measured with IOI method.

Molecule	MW	Neocortex			
		Agarose Gel	AQP4 ^{+/+}	AQP4 ^{-/-}	λ
		D ($10^{-7}\text{cm}^2\text{s}^{-1}$)	D^* ($10^{-7}\text{cm}^2\text{s}^{-1}$)	D^* ($10^{-7}\text{cm}^2\text{s}^{-1}$)	
AF	547	51.70 $\pm$ 1.72 (26)	20.09 $\pm$ 1.55 (29)	20.50 $\pm$ 1.08 (28)	1.59 $\pm$ 0.04
dex3	3,000	22.31 $\pm$ 2.41 (16)	7.25 $\pm$ 0.53 (30)	7.28 $\pm$ 0.81 (40)	1.76 $\pm$ 0.09
dex75	75,000	4.73 $\pm$ 0.18 (33)	0.89 $\pm$ 0.02 (14)	0.90 $\pm$ 0.01 (15)	2.30 $\pm$ 0.15

Data are expressed as mean $\pm$ SD. Total number of records is shown in parenthesis.

Diffusion coefficients were obtained at, or corrected to, 34°C.

$$\lambda = (D/D^*)^{1/2}.$$