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Diffusional Water Permeability (P_{DW}) of Adult and Neonatal Rabbit Renal Brush Border Membrane Vesicles

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Abstract

We have shown that there is a maturational increase in osmotic water permeability (P_f) of rabbit renal brush border membrane vesicles (BBMV). The purpose of the present study was to further investigate the changes in proximal tubule water transport that occur during postnatal development. Diffusional water permeability (P_{DW}) has not been measured directly in adult or neonatal BBMV. We validated the method described by Ye and Verkman (Simultaneous optical measurement of osmotic and diffusional water permeability in cells and liposomes. *Biochemistry* **28**:824-829, 1989) to measure P_{DW} in red cell ghosts and liposomes, to examine the maturational changes in P_{DW} in BBMV. This method utilizes the sensitivity of 8-amino-naphthalene-1,3,6-trisulfonic acid (ANTS) fluorescence to the D_2O - H_2O content of the solvent. ANTS-loaded neonatal (11 days old) and adult BBMV were rapidly mixed with two volumes of isoosmotic D_2O solution using a stopped-flow apparatus at 5°–37°C. P_{DW} was lower in neonatal than adult BBMV at 5° (3.77 ± 0.34 vs. 5.35 ± 0.43 $\mu\text{m/sec}$, respectively, $p < 0.05$) and 20°C (7.03 ± 0.40 vs. 9.04 ± 0.25 $\mu\text{m/sec}$, respectively, $p < 0.001$), but was not different at 30° and 37° C. The activation energy (E_a) was higher in neonatal than in adult BBMV (9.29 ± 0.56 kcal/mol vs. 6.46 ± 0.56 kcal/mol, $p < 0.001$). In adult BBMV, P_{DW} was inhibited by 0.5 mM $HgCl_2$ by $46.6 \pm 3.6\%$, while it was not affected in neonatal BBMV ($p < 0.001$). The results indicate that P_{DW} can be measured in rabbit renal BBMV. There are significant changes in water transport across the apical membrane during postnatal development, consistent with a maturational increase in channel-mediated water transport.

Keywords

Stop-flow kinetics; Development; Fluorescence; Membrane transport

Introduction

Water transport across biological membranes is a fundamental process in cell physiology. Water traverses cell membranes across the lipid bilayer or through water channels, termed aquaporins (Agre et al., 1993). These two distinct pathways are different in nature and have their own characteristics. Channel-mediated water transport has a low activation energy (E_a) and in general is inhibited by mercurials, while transport through the lipid bilayer has a high E_a and is not inhibited by mercury (Verkman, 1989; Verkman et al., 1996).

The pathway for water movement can be determined from measurement of osmotic and diffusional water permeabilities (Finkelstein, 1987). Osmotic water permeability (P_f) is the water permeability of a membrane in the presence of an osmotic or hydrostatic pressure gradient. Diffusional water permeability (P_{DW}), on the other hand, is defined as the permeability measured in the absence of a pressure gradient (Finkelstein, 1987; Verkman et al., 1996). For lipid bilayers lacking water channels, the P_f/P_{DW} ratio is near unity since the osmotic gradient creates a water concentration gradient within the bilayer. Hence, water movement occurs by a 'solubility-diffusion' mechanism, identical to simple diffusion of water (Finkelstein, 1987). An osmotic gradient across a water channel drives water movement by establishing a pressure gradient along the length of the channel and is thus not equivalent to the mere diffusion of water through the channel. This leads to a ratio of P_f and P_{DW} greater than unity (Finkelstein, 1987). The P_f/P_{DW} ratios, along with the E_a and mercury sensitivity, provide important information with regard to the qualitative aspects of transmembrane water transport.

Our laboratory has previously investigated functional changes in water transport of the rabbit proximal tubule during development (Quigley et al., 1998, 2000; Quigley & Baum, 1996). We found that P_f of the brush border membrane (BBM) was lower in the neonate than in the adult (Quigley et al., 1998). Furthermore, the E_a was higher and the percentage inhibition by mercury was lower in neonatal brush border membrane vesicles (BBMV) than in adult vesicles. These functional results indicate that water transport in neonatal BBM is less channel-mediated than that of adults. This was supported by the lower abundance of aquaporin-1 protein (AQP1), the primary water channel in the proximal tubule, in neonatal than adult BBM membranes (Quigley et al., 1998). Measurement of P_{DW} , and its ratio to P_f , of the BBM would provide additional information of basic membrane characteristics in the transition from neonate to adult.

P_f can be measured using stopped-flow kinetics (Chen, Pearce & Verkman, 1988; Quigley et al., 1998; Quigley et al., 2000; Soveral, Macey & Moura, 1997; van Heeswijk & van Os, 1986; Verkman, Dix & Seifter, 1985). After rapid mixing with a hyper- or hypotonic solution, shrinkage or swelling of membrane vesicles can be monitored with either light-scattering or fluorescence-quenching methods (Verkman, 1995). Since P_{DW} is measured in the absence of a gradient, this requires the use of labeled water (e.g., D_2O) (Finkelstein, 1987). To monitor the diffusion of labeled water across the membrane, one can use a fluorophore that is sensitive to the D_2O - H_2O ratio of the solvent. One such fluorophore is 8-aminonaphtalene-1,3,6-trisulfonic acid (ANTS) (Carter et al., 1996, 1997; Phillips, Wong & Yeater, 1999; Ye & Verkman, 1989). Its fluorescence is roughly threefold higher with D_2O

as its solvent compared to H₂O (Kuwahara & Verkman, 1988). Although the combination of ANTS fluorescence and stopped-flow kinetics was proven practical over a decade ago (Ye & Verkman, 1989), there have been no reports on the application of this method to determine P_{DW} in renal BBMVs. Therefore, the combined aims of this study were to validate the use of stopped-flow kinetics and ANTS-fluorescence as a method to measure P_{DW} in rabbit renal BBMVs, as well as its applicability to further investigate the developmental changes that occur in BBMVs during postnatal maturation.

Materials and Methods

Animals

Adult New Zealand white rabbits were housed at our institution and fed a standard laboratory chow. They were kept on a 12-12 hr light-dark cycle and at an ambient temperature of 16°–22°C. Pregnant does were housed at least nine days prior to delivery and cared for their neonates. Adult rabbits were greater than 11 weeks of age and neonates were studied at 9–11 days of age.

Brush Border Membrane Vesicle Preparation

Brush border membrane vesicles were prepared as previously described (Quigley et al., 1998). Briefly, animals were sacrificed and the kidneys were removed promptly and placed in ice-cold PBS (in mM: 137 NaCl 2.7 KCl 10.1 Na₂HPO₄ 1.7 KH₂PO₄, pH = 7.4). Kidneys from 3–5 neonates were pooled. The capsule was removed and the cortex was dissected. After mincing, the cortex was put in 15 ml isolation buffer (in mM: 300 D-mannitol, 16 HEPES, 5 EGTA, adjusted to a pH of 7.5 with Tris-HCl containing the protease inhibitors aprotinin (2 µg/ml), leupeptin (2 µg/ml) and phenylmethylsulfonyl fluoride (175 µg/ml)). Tissue was homogenized by 15 strokes with a Potter homogenizer. After addition of 230 µl of 1.0 M MgCl₂ to precipitate cell debris, the homogenate was shaken vigorously for 10 sec every 5 minutes for 20 minutes. Subsequently, the homogenate was centrifuged at: 2,500 × g for 15 minutes at 4°C. The supernatant was decanted, added to 230 µl 1.0 M MgCl₂, shaken vigorously for 10 sec every 5 min for 20 min, and centrifuged for 15 min at 2,500 × g at 4°C. The supernatant was then centrifuged at 48,400 × g for 30 min at 4°C. The pellets were resuspended in 1.5 ml of ice-cold resuspension buffer (5.0 mM HEPES, pH 7.4; osmolality adjusted to 80 mOsm with D-mannitol) using 22- and 25-gauge needles. The protein content was determined in the crude homogenate and BBMVs using BCA protein assay (Pierce, Rockford, IL). Alkaline phosphatase activity was used to determine the enrichment as described previously (Arar, Levi, and Baum, 1994; Quigley et al., 1998). There was no difference between adult and neonatal BBMVs in alkaline phosphatase enrichment (6.6 ± 0.7 vs. 6.0 ± 0.7-fold, respectively, $p = \text{NS}$).

ANTS Loading

BBMV-suspension was brought to a concentration of 10 mg protein/ml and loaded with ANTS (10 mM) in the dark, overnight (12–16 hr) at 4°C. After loading, BBMVs were washed five times with ice-cold 80 mOsm H₂O-resuspension buffer and centrifuged (368,000 × g) for 10 min at 4°C. After the fifth spin, pellets were resuspended using 22- and 25-gauge needles and vesicles were brought to a final concentration of 1.0 mg protein/ml.

Stopped-Flow Kinetics

ANTS-loaded vesicles (100 μ l) were rapidly mixed with 80 mOsm resuspension buffer prepared with D₂O as solvent (200 μ l) using a stopped-flow apparatus (SFM-3, Biologic, France: theoretical dead-time 0.8 msec). ANTS was excited using a 75W xenon arc lamp and a monochromator that was set at 380 nm. Emission was measured using a photomultiplier tube (Biologic, France) equipped with a 500 nm cut-on filter and oriented at a 90° angle with regard to the excitation axis. Data were collected at 100- μ sec intervals for 50 msec using Biokine software (Biologic, France). Five to ten raw tracings were averaged and analyzed subsequently.

Temperature Dependence and Mercury Sensitivity

Experiments were conducted at 5, 20, 30 and 37°C. Vesicles were kept on ice at all times to limit ANTS leakage (Ye & Verkman, 1989). Inhibition of water transport across water channels was studied by incubating BBMV with 0.5 mM HgCl₂ at room temperature (~23°C) for 10 min and measuring P_{DW} at 20°C.

Calculation of P_{dw}

Averaged tracings were normalized to initial fluorescence and fitted the following single-exponential curve using Biokine Software (Molecular Kinetics, Pullman, WA):

$$I(t) = I_{\max}(1 - e^{-ky})$$

where $I(t)$ is the fluorescence intensity at time t , $I_{\max}$ is the maximum intensity at infinite time, k is the rate constant and t is time.

P_{DW} was then calculated using the following equation:

$$P_{DW} = 1 / [\tau_{\text{ex}}(S/V)],$$

where τ_{ex} is the exchange time ($1/k$), and S/V the surface to area ratio of BBMV (Lawaczeck, 1984). Our laboratory has previously measured the diameter of adult and neonatal BBMV using electron microscopy. The diameter was 202.2 ± 6.7 nm for the adult BBMV and 192.0 ± 5.1 nm for the neonatal BBMV ($p = \text{NS}$, $n = 210$ for adult BBMV and $n = 220$ for neonatal BBMV) (Quigley et al., 1998). A value of 200 nm was used for the present calculations of P_{DW} .

E_a was calculated from the slope of Arrhenius plots ($\ln k$ vs. temperature^{-1}) using linear regression analysis and the equation:

$$\ln(K_1/K_2) = - [E_a/R]^* [1/T_1 - 1/T_2],$$

where K_i is the rate constant at temperature T_i and R the gas constant ($1.987 \text{ cal deg}^{-1} \text{ mol}^{-1}$) (Berry, 1985).

Materials

Chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and Fluka BioChemika (Buchs, Germany). ANTS was obtained from Molecular Probes (Eugene, OR).

Statistical Analysis

Data were analyzed using Sigma Stat statistical software (Jandel Corporation, San Rafael, CA). Data are presented as mean $\pm$ SEM, unless otherwise noted. Comparisons were performed using independent samples t -tests or Mann–Whitney tests, whichever was appropriate.

Results

Validation of the Method

Several experiments were conducted to validate the methods. We first assessed if it was possible to mix BBMV with D₂O-resuspension buffer at a rate fast enough to measure P_{DW} . ANTS (10 mM) was dissolved in H₂O-resuspension buffer and mixed with 2 volumes of D₂O-resuspension buffer. This concentration of ANTS corresponds to the intravesicular ANTS concentration of loaded BBMV. The resulting fluorescence signal was time-independent over a temperature range of 5°–37°C, which is consistent with earlier reports (Kuwahara et al., 1988; Ye & Verkman, 1989). In Fig. 1, tracings demonstrating the mixing of ANTS and D₂O-resuspension buffer are shown together with those obtained with intact, adult BBMV. The latter are both time- and temperature-dependent. To determine if this difference could be an artifact due to the higher viscosity of BBMV-suspensions, ANTS was allowed to leak from loaded BBMV for > 48 hr at room temperature followed by three cycles of snap-freezing (liquid nitrogen) and thawing (37°C waterbath) to fully disrupt the membranes. The rate constant for these suspensions was much higher than for intact BBMV ($585 \pm 63 \text{ sec}^{-1}$ vs. $271 \pm 23 \text{ sec}^{-1}$; $p < 0.005$). Addition of 0.5 mM HgCl₂ did not alter the rate of change in fluorescence of 10 mM ANTS, as it remained time-independent (*not shown*).

Developmental Changes in P_{DW}

BBMV were prepared from 9 adult rabbits and 8 sets of neonates. Figures 2a and 2b show typical tracings for adult and neonatal BBMV. As shown in Fig. 3a, P_{DW} was higher in adult BBMV than in neonatal BBMV at 5°C and 20°C. At 30° and 37°C, there was not a significant difference in P_{DW} between adult and neonatal BBMV. E_a , obtained from an Arrhenius plot (Fig. 3b), was lower in adult than in neonatal BBMV (adult $6.46 \pm 0.56 \text{ kcal/mol}$ vs. neonate $9.29 \pm 0.56 \text{ kcal/mol}$, $p < 0.001$). Both the lower P_{DW} -values at low temperatures and the greater temperature dependence in neonatal BBMV are consistent with less channel-mediated water movement in neonatal BBMV than in adults, as demonstrated in our previous study comparing P_f in adult and neonatal BBMV (Quigley et al., 1998).

Addition of 0.5 mM HgCl₂ to BBMV affected both the rate constant as well as the amplitude of the fluorescence signal (Fig. 2a and 2b). The latter was consistently lower in the presence

of HgCl_2 for both adult and neonatal BBMV. This led us to believe that for the proper analysis of the effects of mercury on P_{DW} , we needed to take both changes into consideration and we therefore compared the initial slopes. The initial slope of the single-exponential curve is equal to the product of its amplitude and rate-constant. As shown in Fig. 4, the inhibition of P_{DW} was found to be $46.6 \pm 3.6\%$ in adults and $-4.8 \pm 7.6\%$ in neonates ($p < 0.001$). This indicates that water movement is channel-mediated to a far greater extent in adult BBMV than in those from neonates. The diffusional movement of water across the neonatal brush border membrane is not affected by HgCl_2 and is thus predominantly through the lipid bilayer.

Discussion

This study demonstrates that P_{DW} can be measured in rabbit renal BBMV using fluorescence and stopped-flow kinetics. Rapid mixing of ANTS-loaded vesicles with two volumes of D_2O gave a reproducible, time-dependent signal. In intact BBMV, the change in fluorescence was much slower than that of ANTS-containing resuspension buffer mixed with D_2O , demonstrating that the time resolution of the method was sufficiently accurate to measure P_{DW} . Moreover, when the fluorophore was allowed to leak out of the BBMV, the rate at which the change in fluorescence occurred was greater than that in intact BBMV, indicating that the higher viscosity of the vesicle suspension was not an artifact in these measurements. The present study demonstrates for the first time also that P_{DW} of adult BBMV is higher at lower temperatures (5°C and 20°C) than neonatal BBMV and E_a is lower than that of neonatal BBMV. In addition, the presence of HgCl_2 reduces P_{DW} roughly by half in adult BBMV, yet has no effect in neonatal BBMV. These results indicate that the movement of water through the adult BBM is channel-mediated to a greater extent than that in neonatal BBM.

Previously, P_{DW} had not been measured directly in the isolated renal BBMV, although two separate groups of investigators used a proton NMR-technique to measure P_{DW} of plasma membranes of isolated proximal tubule cells (Carpi-Medina et al., 1988; Meyer & Verkman, 1987). With this method, the relaxation of magnetized water protons is monitored. Mn^{2+} is used as an impermeant paramagnetic quencher that accelerates the proton relaxation of extracellular water. The rate of the slower intracellular relaxation is dependent on the rate of diffusion of labeled water molecules across the cell membrane and is measured by NMR. P_{DW} was $22.3 \mu\text{sec}$ (at 23°C) and $32 \mu\text{sec}$ (at 37°C) after correction for the membrane surface area (Carpi-Medina et al., 1988; Meyer et al., 1987). E_a and the mercury inhibition of P_{DW} were $5.2 \pm 1.0 \text{ kcal/mol}$ and 55% , respectively (Carpi-Medina et al., 1988). The reported values for P_{DW} are roughly 1.5–2.5-fold higher than those presented in this study. The proximal tubule cells contained both the BBM and basolateral membranes (BLM), but it is unclear whether this can explain the observed difference in P_{DW} . P_f has been found to be both higher and lower in BLM than BBM (Meyer et al., 1987; Quigley et al., 2000; van Heeswijk et al., 1986). Furthermore, P_{DW} of the BLM, measured in proximal tubule suspensions using NMR, was found to be lower ($20 \mu\text{m/sec}$ (Verkman & Wong, 1987)) than the previously reported P_{DW} of isolated cells (Carpi-Medina et al., 1988; Meyer et al., 1987). Hence, it is unclear what accounts for the difference in P_{DW} measured in BBMV in our study and in that in isolated proximal tubule cells measured by others. The maturation of

P_{DW} in human red blood cells has also been studied using this technique (Benga et al., 2001). The findings were similar to our findings in that the P_{DW} of the neonatal RBCs was lower than the adult RBCs but the activation energy for P_{DW} was higher in the neonatal RBCs.

Another approach to estimate P_{DW} was to use in vitro microperfusion of isolated proximal tubules (Berry, 1985). The diffusional permeability coefficients of [^3H] water and n -[^{14}C] butanol were measured simultaneously in perfused proximal convoluted tubules. P_{DW} of the two membranes in series was estimated based on the assumptions that the epithelial permeability of n -butanol was limited by the cytoplasmic compartment due to its high partition coefficient in lipid bilayers and that its diffusional characteristics in cytoplasm are similar to water. Thus, the difference of measured, epithelial permeabilities for water and n -butanol can be attributed to the resistance of the apical and basolateral membranes to water. P_{DW} for the brush border and basolateral membranes in series was $124.7 \pm 0.7 \mu/\text{sec}$ (39°C) with an E_a of 4.3 kcal/mol (Berry, 1985). The P_{DW} was inhibited by 54% with mercury. Assuming that both membranes contribute equally to P_{DW} , this would result in a P_{DW} as high as 250 $\mu\text{m}/\text{sec}$ for the apical membrane. In addition, the P_{DW} obtained from in vitro microperfusion is a minimum due to possible unstirred-layer effects (Barry & Diamond, 1984; Berry, 1985). More importantly, in these calculations the luminal surface area of the proximal tubule was assumed to be a smooth cylinder, while in reality, this is not the case. Microvilli increase the actual surface area 15-fold (proximal straight tubule) to 36–39-fold (proximal convoluted tubule) (Evan, Gattone & Schwartz, 1983; Welling & Welling, 1975). Correction for this yields a value for P_{DW} of the proximal tubule BBM (6.4–16.7 μm) that is similar to that obtained in the present study.

The combination of ANTS fluorescence and stopped-flow kinetics had previously been used to measure P_{DW} in human red cell ghosts and liposomes (Ye & Verkman, 1989). In human red cell ghosts, which are known to be highly permeable due to high AQP1 expression, P_{DW} was 50 $\mu\text{m}/\text{sec}$ (at 25°C) (Ye & Verkman, 1989). This was consistent with other reported measurements (Conlon & Outhred, 1972; Martial & Ripoché, 1991) and considerably higher than the values for BBMV as presented in this study. Interestingly, mercury inhibition was found to be 45% (Ye & Verkman, 1989), which is very similar to the percent inhibition in adult BBMV in the present study. Apparently, the proportion of channel-mediated water movement in human red cell ghosts and adult BBMV is similar. On the other hand, P_{DW} was lower (6.3 $\mu\text{m}/\text{sec}$ at 23°C ; Ye & Verkman, 1989) in phosphatidylcholine/cholesterol (90%/10%) liposomes than in BBMV. This value is only slightly lower than that for neonatal BBMV found in the present study.

The maturational increase in P_{DW} is consistent with the maturational changes of P_f (Quigley et al., 1998) and the postnatal increase in expression of AQP1 (Bondy et al., 1993; Devuyst et al., 1996; Smith et al., 1993; Yamamoto et al., 1997). This study also provides additional evidence that water movement is channel-mediated to a greater extent in adult than in neonatal BBMV. Using the P_f measured in our previous study (Quigley et al., 1998), the P_f/P_{DW} ratio for adult BBMV at 20–25 $^\circ\text{C}$ was ~ 10 , compared to only ~ 6.5 for neonatal BBMV. In addition, the temperature dependence of water movement, as reflected by E_a , was comparable for P_f and P_{DW} in both adult and neonatal BBMV. For P_f , E_a was 5.09 ± 0.57

kcal/mol in adult BBMV and 9.19 ± 0.37 kcal/mol in neonatal BBMV (Quigley et al., 1998), whereas in the present study the E_a for PDW was 6.46 ± 0.56 kcal/mol and 9.29 ± 0.56 kcal/mol, respectively. E_a is high (> 10 kcal/mol) for transport through the lipid bi-layer and low (< 6 kcal/mol) for transport through aquaporins (Verkman et al, 1996). Therefore, the obtained values for E_a further support more channel-mediated water transport in the adult renal BBM.

In the present study P_{DW} was inhibited by 0.5 mM HgCl₂ in adult BBMV, but HgCl₂ had no effect on neonatal BBMV. The estimated P_f/P_{DW} ratio (~ 6.5) suggests that water movement through the neonatal BBM is channel-mediated to some extent. Furthermore, we have previously shown that AQP1 is expressed in the BBM of 10–14-day old rabbits and that 1 mM HgCl₂ inhibits P_f by $17.9 \pm 1.3\%$ in neonatal BBMV (Quigley et al., 1998). It is unclear why HgCl₂ did not inhibit P_{DW} in the present study. It is possible that the neonatal membrane has an additional aquaporin that has a lower sensitivity to mercury.

Although P_{DW} was lower in neonatal than in adult BBMV at 5° and 20°C, it was not lower and even tended to be slightly higher at 30° and 37°C. This is consistent with our previous observation that P_f in neonatal BBMV was not different from that in adult BBMV at higher temperatures (Quigley et al., 1998). The fact that the neonatal P_f and P_{DW} were comparable to adult BBMV at higher temperatures is likely due to changes in the membrane fluidity (Medow & Segal, 1987). The fluidity in the rat and rabbit BBM decreases during maturation (Medow & Segal, 1987), which probably reflects a combination of changes in lipid and protein composition of the BBM (Arar et al., 1994). Increases in P_f of apical membrane vesicles prepared from bovine tracheal epithelium have been linked directly to increased membrane fluidity (Worman et al., 1986). In the intestinal BBM, fluidity was affected by maturational changes in cholesterol-phospholipid content (Schwarz et al., 1984). Nonetheless, protein-lipid interactions were considered to be the major determinants, because fluidity was not different between adults and neonates in protein-free, reconstituted liposomes (Schwarz et al., 1985).

In conclusion, the present study shows that the combination of stopped-flow kinetics and ANTS-fluorescence can be used to measure P_{DW} in rabbit renal BBMV. Its applicability was illustrated by the results indicating that the developmental changes in the proximal tubule include the increase in contribution of channel-mediated water transport through the BBM. This also indicates that, due to the known high degree of water permeability in this section of the nephron, the described method will most likely be of use in further studies of P_{DW} in membranes in other sections of the nephron as well as other tissues.

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Fig. 1.

Comparison of fluorescence signals from intact, ANTS-loaded adult BBMV and ANTS solution (10 mM) mixed with two volumes of iso-osmotic D₂O-resuspension buffer at 5°, 20° and 37°C. ANTS fluorescence increased as the D₂O-H₂O ratio of its solvent composition increased. Tracings were normalized to initial fluorescence and fitted to a single-exponential curve. For ANTS solution, the signal was time-independent at all temperatures. For BBMV, fluorescence was not time-independent and the rate of change increased at higher temperatures. This shows that the rate of mixing was sufficiently rapid to measure P_{DW} in BBMV.

Fig. 2.

Typical tracings (averaged raw tracing and single-exponential fit) for adult (*a*) and neonatal (*b*) BBMVs at 20°C. Curves were normalized to initial fluorescence. The bottom curves were obtained after incubation with 0.5 mM HgCl₂ at room temperature for ~20 min. HgCl₂ reduced the initial slope by $46.6 \pm 3.6\%$ in adult BBMVs, but had no effect on neonatal BBMVs.

Fig. 3.

Temperature dependence of P_{DW} of adult and neonatal BBMVs. (a) P_{DW} of neonatal BBMVs was lower at 5° and 20° C. (b) Arrhenius plot. E_a was calculated from the slope of the regression lines. E_a was 6.46 ± 0.56 kcal/mol in adult BBMVs and 9.29 ± 0.56 kcal/mol in neonatal BBMVs ($p < 0.001$). * $p < 0.02$, ** $p < 0.001$ for both *a* and *b*.

Fig. 4.

Mercury sensitivity of P_{DW} in adult and neonatal BBMVs at 20°C. BBMVs were incubated with 0.5 mM HgCl_2 at room temperature for ~20 min before the experiment. The percentage inhibition of P_{DW} was calculated from the decrease in initial slope of the fitting single-exponential curve. * $p < 0.001$ vs. adult BBMVs.