J Membrane Biol (2010) 236:225–232
DOI 10.1007/s00232-010-9295-9

Stimulating Effect of External *Myo*-Inositol on the Expression of Mutant Forms of Aquaporin 2

Yoann Lussier · Pierre Bissonnette · Daniel G. Bichet · Jean-Yves Lapointe

Received: 13 May 2010 / Accepted: 20 July 2010 / Published online: 14 August 2010

Abstract *Myo*-inositol (MI; hexahydroxycyclohexane, $C_6H_6O_{12}$) is a small neutral molecule used as a compatible osmolyte in the kidney medulla. At high concentrations, MI appears to act as a chemical chaperone and was shown to promote plasma membrane expression of the impaired cystic fibrosis chloride channel (Δ508-CFTR). In the present study, we measured whether MI could increase expression of two human aquaporin 2 (AQP2) mutants which were recently identified as causing nephrogenic diabetes insipidus (NDI). Both proteins (D150E and G196D) were expressed in *Xenopus laevis* oocytes, but only D150E displayed an increase in oocyte water permeability (P_f). Adding 5 mM MI to the bathing solution for 24 h produced a 50% increase in the D150E-associated P_f, while it had no effect on noninjected oocytes or on oocytes expressing wt-AQP2 or G196D. Western blots performed on purified plasma membrane preparations confirmed that MI increased the amount of D150E present at the plasma membrane, while G196D was always undetectable. *X. laevis* oocytes are remarkably impermeable to MI, and the effect of MI on D150E expression does not require the presence of intracellular MI. The effect of external MI was dose-dependent ($K_{0.5}$ was 130 μM) and specific with respect to other forms of inositols. Further studies on a second group of AQP2 mutants causing NDI showed that K228E activity was similarly stimulated by MI, while V71M, A70D and S256L were not. It is concluded that physiological concentrations of extracellular MI can stimulate the expression of a specific subgroup of AQP2 mutants.

Keywords Aquaporin · Protein expression · *Myo*-inositol · Nephrogenic diabetes insipidus · Chemical chaperone · AQP2

Y. Lussier · P. Bissonnette · D. G. Bichet · J.-Y. Lapointe (✉)
Groupe d'étude des protéines membranaires (GÉPROM),
Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montreal,
QC H3C 3J7, Canada
e-mail: jean-yves.lapointe@umontreal.ca

Introduction

Aquaporin-2 (AQP2) is the vasopressin-dependent water channel of the renal collecting duct which mediates the final water reabsorption in the kidney. Impaired AQP2 function is responsible for the autosomal form of nephrogenic diabetes insipidus (NDI). It is thought that most NDI-inducing AQP2 mutations generate altered protein variants that are misprocessed, retained in the endoplasmic reticulum (ER) and destined for degradation through ER-associated protein degradation (ERAD) (Bonifacino and Weissman 1998). Mistargeting of mutated membrane proteins such as channels and receptors is responsible for a large number of pathologies known as "conformational diseases" (Schröder and Kaufman 2005). These include various types of neurological and metabolic disorders as well as several channelopathies including cystic fibrosis (CF). Utilization of chemical chaperones (Welch and Brown 1996) like trimethylamine *N*-oxide, taurine and their derivatives to rescue defective proteins has been the focus of several studies (Tamarappoo and Verkman 1998; Ozcan et al. 2006). At high concentrations, *myo*-inositol (MI) can act as a chemical chaperone and augment proper protein folding (McLaurin et al. 2000; Mishra et al. 2007). MI is also a compatible osmolyte used by cells exposed to harsh conditions, such as the hypertonic milieu of the renal medulla, in order to support efficient protein synthesis (Bagnasco et al. 1986). While

plasma MI does not exceed 50 μM (Holub 1986), intracellular MI may reach values as high as 30 mM in renal medullary cells as well as in brain cells (Novak et al. 1999; Fisher et al. 2002). This was the rationale for a study where fibroblastic 3T3 cells expressing the Δ508-CFTR chloride channel (the most common CF-causing mutant) were exposed for 24 h to a hypertonic solution containing 300 mM MI (Howard et al. 2003). Under these conditions, the expression level of Δ508-CFTR and cell conductance were significantly increased compared to untreated cells. A similar study was published by Zhang et al. (2003) using IB3 cells expressing Δ508-CFTR in the presence of 10 mM MI alone or in conjunction with other compatible osmolytes such as betaine and taurine. The addition of these compatible osmolytes was shown to approximately double the membrane expression of Δ508-CFTR, which would normally be largely retained in the ER. Even though no mechanism of action has been proposed, these studies indicate that prolonged exposure to high MI concentrations enhanced the processing of the mutated protein and, somehow, increased its targeting to the plasma membrane.

CFTR and AQP2 share similarities as both are plasma membrane channels bearing naturally occurring mutations that essentially impede the normal protein maturation process in the ER and beyond. In the present study, we investigated the effect of MI on the membrane expression of two AQP2 mutants which were recently identified as causing NDI in a family of Spanish origin (Guyon et al. 2009). Our results show that the G196D mutation generates an AQP2 variant that is completely retained within intracellular stores and thus displays no functionality, while the D150E mutant is weakly expressed at the plasma membrane with compromised functionality ($\sim$1/20 of wild-type [wt] function). We now report that a low external MI concentration (0.5 mM) was sufficient to stimulate water permeability due to D150E expression and, surprisingly, this effect did not require MI transport into the cell. The observation of a strictly extracellular MI effect on the expression of a misfolded protein is of considerable physiological interest per se, and further studies will be required to discover the nature of the underlying mechanism. The aim of the present study was to characterize the stimulating effect of extracellular MI on misfolded protein expression, with respect to its sensitivity and its specificity for different target proteins and for different inositol isomers.

Materials and Methods

Oocyte Preparation and Incubation

Ovarian tissue from *Xenopus laevis* (University of Alberta, Edmonton, Canada) was surgically removed under tricaine anesthesia, and stage V–VI oocytes were manually separated and then defolliculated by collagenase digestion as described earlier (Bissonnette et al. 1999). Oocytes were injected with water or with water + mRNA encoding either a wt (1 ng) or a mutant (10 ng in each case) form of AQP2. They were then incubated at 18°C in Barth's solution (mM: 90 NaCl, 3 KCl, 0.82 $MgSO_4$, 0.41 $CaCl_2$, 0.33 $Ca[NO_3]_2$, 5 HEPES, pH 7.6) supplemented with 5% horse serum, 2.5 mM sodium pyruvate, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin and 0.1 mg ml^{-1} kanamycin for a 24-h incubation period (post-mRNA injection) in the presence of the indicated concentration of MI or mannitol. All procedures regarding *X. laevis* treatments and manipulations were performed in accordance with Canadian guidelines and the ethics committee of the Université de Montréal. Also, when required, animals were killed by prolonged anesthesia.

Vectors, mRNA Preparation and Injection

Naturally occurring mutations in AQP2 (A70D, V71M, D150E, G196D, K228E and S256L) (Marr et al. 2002; Cheong et al. 2005; McDill et al. 2006; Guyon et al. 2009) were created through site-directed mutagenesis of pT7TS-AQP2wt as described previously (Guyon et al. 2009). All constructs were sequenced to verify that the correct mutation had been encoded. Plasmids were linearized, and capped mRNA was synthesized in vitro using the mMessage mMachine kit (Ambion, Austin, TX) according to the manufacturer's instructions.

Volume Measurements

Functional activity of AQP2 variants was measured using a volumetric measurement apparatus as previously described (Duquette et al. 2001). Briefly, a glass-bottomed chamber of 120 μl was perfused at a rate of $\sim$1.5 ml min^{-1} and illuminated from above by a light-emitting diode touching the surface of the solution. Solution changes were under the control of an electrical valve with a characteristic dead space of 50 μl. The oocyte cross section was continuously measured from below with a CCD camera and a 3× objective using custom-made software. Values were taken at a rate of either 5 or 25 times per second, and data were averaged over 1-s periods. Oocyte volume was obtained assuming spherical symmetry and calibrated with a steel ball of a precisely known volume. Cell swelling (dV/dt) was measured using a 20 mOsm hypo-osmotic shock and compared to initial volume variations recorded immediately before application of the hypo-osmotic medium (dV_0/dt). Water permeability (P_f, in cm per second) was calculated using the following equation:

$$P_f = \frac{1}{S \times v_w \times \Delta\pi}\left(\frac{dV}{dt} - \frac{dV_0}{dt}\right) \quad (1)$$

where S is a standardized total oocyte membrane area of 0.4 cm^2 per oocyte (including the infolding factor), v_w is the water-specific volume (18.2 cm^3 mol^{-1}) and $\Delta\pi$ is the amplitude of the hypotonic shock applied (20 mOsmol/1,000 cm^3).

Solutions

Solutions for volumetric experiments contained (in mM) 80 NaCl, 3 KCl, 0.82 $MgCl_2$, 0.74 $CaCl_2$, 25 mannitol and 5 mM HEPES (pH 7.6). Hypo-osmotic solutions were made by omitting 20 mM mannitol from solution, while the addition of MI was compensated by equimolar removal of mannitol. Variations in solution osmolalities were kept within ±2 mOsm kg^{-1} H_2O and verified using a freezing-point osmometer (Advanced DigiMatic Osmometer, model 3D2; Advanced Instruments, Needham Heights, MA).

Radiotracer Experiments

For experiments where the uptake of MI was measured, we used Barth's solution containing 10 μM cold MI along tracer (1 μCi/μl [2-^{3}H] MI). Groups of 10–12 oocytes were first rinsed in substrate-free Barth's solution and exposed to the transport solution for 1 h. Oocytes were then washed three times in substrate-free Barth's solution and placed in separate scintillation vials, where they were dissolved in 200 μl 10% SDS for at least 2 h. Five milliliters of scintillation cocktail (Beta Blend; ICN, Irvine, CA) was then added, and the vials were assayed for ^{3}H activity with an LS6000 SC scintillation counter (Beckman, Fullerton, CA).

Western Blot

Total membranes and purified plasma membranes were produced using a method recently described by this laboratory (Leduc-Nadeau et al. 2007). Western blots for total membrane protein were performed by loading the material obtained from two oocytes on a 12% polyacrylamide gel. In the case of purified plasma membrane proteins, the lower yield was compensated for by using material purified from 40 oocytes. After migration, the proteins were transferred onto nitrocellulose membranes, blocked in TBS-T containing 5% nonfat milk and incubated overnight at 4°C with a goat anti-AQP2 (C-17, at 1/500 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were later rinsed, blocked and reprobed for 1 h at room temperature with the secondary antibody (HRP-linked chicken anti-goat, 1/10,000 dilution; Santa Cruz Biotechnology) in milk-supplemented TBS-T. Revelation was achieved by enhanced chemiluminescence detection (Phototope-HRP; New England Biolabs, Pickering, Canada). Molecular weights were determined and densitometric analyses were performed using software from Alpha-Imager 2000 (Alpha Innotech, San Leandro, CA).

Data Analysis and Statistics

Figures showing P_f measurements represent typical experiments done with 8–12 oocytes per data point (mean ± SE). The experiments were repeated three to five times with oocytes obtained from different donors. Significance was tested by one-way ANOVA with a Bonferroni test, followed by a post hoc multiple means comparison test or by an unpaired Student's *t*-test; statistical significance was set at $P < 0.05$ (unless otherwise mentioned).

Results

Figure 1 illustrates a typical experiment where water permeabilities (P_f) were measured for several groups of oocytes coming from a single donor frog (each bar representing the average P_f obtained from 10 oocytes). Oocytes were injected with mRNA coding for either wt-AQP2 (1 ng), D150E or G196D mutants (10 ng) and incubated for 24 h, along with control noninjected oocytes, in the presence or absence of 5 mM MI (mannitol replacement). In the absence of MI, wt-AQP2-expressing oocytes were characterized by a high P_f (22.3 ± 1.9 × 10^{-4} cm s^{-1}), which was four times larger than the P_f of noninjected

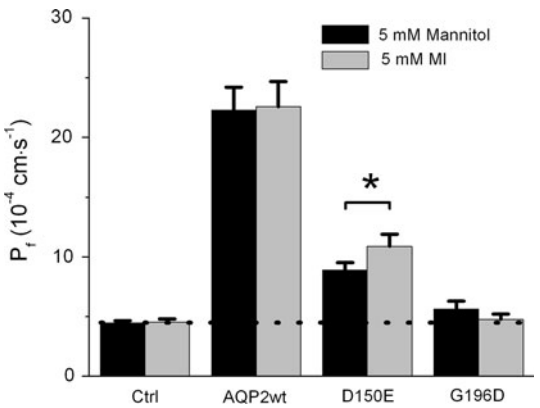

Fig. 1 Effect of 5 mM MI on the activity of wild-type and two mutant forms of AQP2. Each column represents the average water permeability (P_f, ± SE) measured in 10 oocytes from a single donor frog. *Gray* and *black bars* represent permeabilities measured from oocytes which have experienced 24-h incubation with 5 mM MI or with 5 mM mannitol, respectively. * Statistically significant difference between samples treated with the two different polyols ($P < 0.05$)

oocytes ($4.47 \pm 0.18 \times 10^{-4}$ cm s^{-1}). In agreement with previous observations from this laboratory (Guyon et al. 2009), oocytes expressing D150E displayed a moderate P_f ($8.90 \pm 0.61 \times 10^{-4}$ cm s^{-1}), while G196D expression gave a P_f that was not significantly different from the P_f of noninjected oocytes ($5.63 \pm 0.67 \times 10^{-4}$ cm s^{-1}). As seen in Fig. 1, the presence of 5 mM MI in incubation medium for 24 h stimulated P_f in oocytes expressing D150E (an average P_f of $10.9 \pm 0.1 \times 10^{-4}$ cm s^{-1}) but not in the three other groups of oocytes. When considering specific activities (by subtracting the basal P_f value of noninjected oocytes of the same donor), the P_f associated with the expression of D150E in the data shown was stimulated by 45% in the presence of MI. The average stimulation ($n = 3$) for experiments of the type shown in Fig. 1 of the D150E-specific P_f was $55 \pm 13\%$.

One strategy to promote MI entry into oocytes is to express the Na^+/MI transporter SMIT2, a concentrative transport system for MI (Coady et al. 2002). As seen in the inset to Fig. 2a, incubating SMIT2-expressing oocytes for 1 h in the presence of 10 μM MI generated MI uptake of 19.4 ± 3.3 pmol/oocyte. In contrast, the measured uptake in noninjected oocytes was 1,000 times smaller (0.024 ± 0.014 pmol) and mostly represents the nonspecific binding to the oocyte surface as this value was not displaced by excess cold MI (data not shown). Similar results were obtained for three different batches of oocytes, and we can conclude that noninjected oocytes are basically impermeable to MI. In order to see whether an increase in intracellular MI could contribute to the observed stimulation of D150E activity, P_f measurements were performed on oocytes expressing SMIT2 (10 ng mRNA) alone or in coexpression with the various forms of AQP2. As shown in Fig. 2a, SMIT2 is associated with a certain passive water permeability, as has previously been reported for the closely related Na/glucose cotransporter SGLT1 (Loo et al. 1996; Duquette et al. 2001). Using this new P_f as a baseline, the effect of 24-h incubation with 5 mM external [MI] can be seen to not differ from the effect observed in the absence of SMIT2. On average, for four experiments of the type presented in Fig. 2a, the P_f associated with D150E was increased $83 \pm 31\%$ by 5 mM external [MI] in the presence of SMIT2, which is not significantly different from the stimulation observed in the absence of SMIT2.

These data indicate that the stimulatory effect of MI on D150E membrane expression cannot be enhanced by increasing MI uptake. To further test the possibility that MI is not acting from the intracellular milieu, the effect of intracellular MI was assayed through direct microinjection of MI. Oocytes were thus injected with 10 ng of D150E mRNA in 46 nl of pure water or 46 nl of a 200-mM MI solution. With an estimated accessible volume of 500 nl (Lapointe et al. 2002), the final MI concentration within the

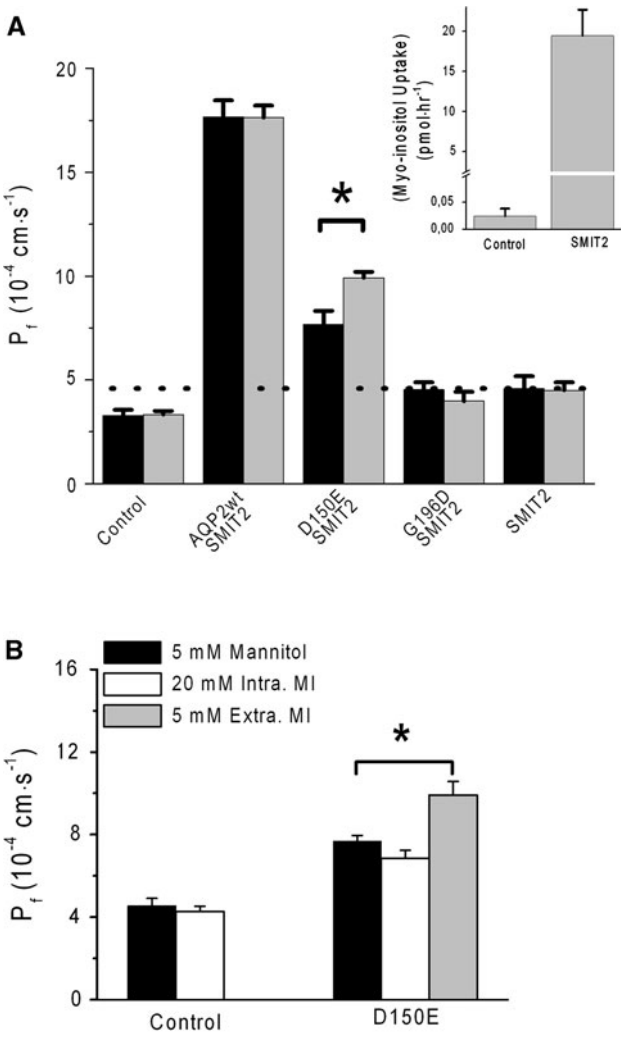

Fig. 2 Absence of effect of intracellular MI on the water permeability (P_f) associated with AQP2 variants. **a** Effect of incubation with 5 mM MI (*gray bars*) or 5 mM mannitol (*black bars*) on the P_f of oocytes coexpressing different forms of AQP2 with SMIT2, a secondary active Na/MI cotransporter. The oocyte P_f was significantly larger in the presence of SMIT2 vs. noninjected oocytes ($P < 0.005$, $n = 8$ oocytes in each group). The stimulation due to the presence of MI was significant only for the case of D150E ($P < 0.005$, $n = 8$ oocytes). *Inset* shows that MI uptake is 1,000 times larger in oocytes expressing SMIT2 than in noninjected oocytes (10 oocytes in each group, see text for experimental details). **b** Injecting oocytes with 46 nl of a 200 mM MI solution produced no significant changes in the P_f of control oocytes or in D150E-expressing oocytes. In contrast, exposing D150E-expressing oocytes to 5 mM external MI produced a significant P_f increase in this series of experiments ($P < 0.005$, $n = 8$ oocytes in each group)

oocyte is estimated to be 20 mM, a value similar to that reached using SMIT2 in the presence of 0.5 mM external MI (data not shown). As shown in Fig. 2b, intracellular MI injection completely failed to mimic the effect of extracellular MI on the function of the D150E mutant. In the example shown, extracellular MI (5 mM) stimulated D150E-specific P_f by 72% ($P < 0.05$), while intracellular

MI injection inhibited this P_f by 26% (not significant, $P = 0.4$). Also shown in Fig. 2b, the presence of intracellular MI does not affect the endogenous oocyte P_f as values are similar in control oocytes injected either with water or with 200 mM MI solution (46 nl).

During the course of the present study, the stimulatory experiment presented in Fig. 1 was repeated with 25 different oocyte donors, which can be used to obtain a statistical distribution of the effect of MI on the specific activity of D150E. As presented in Fig. 3a, the level of stimulation due to MI ranged 10–100% and can be fitted with a gaussian distribution curve characterized by a mean stimulatory effect of 50% and a standard deviation of 22%.

To further characterize the MI stimulatory action on D150E, assays looking at both the sensitivity and specificity of the MI action were performed. As shown in Fig. 3b, exposing D150E-expressing oocytes to various MI concentrations (0–400 μM) for 24 h induced specific P_f increases, which were used to generate a typical dose–response curve exhibiting an ED_{50} of 130 ± 15 μM. This experiment was repeated three times, and ED_{50} values were found to vary between 100 and 200 μM.

In principle, the effect of MI on the membrane activity of D150E must either be due to a stimulation of the number of water channels expressed at the plasma membrane or to a modification of the water permeability of each water channel present. To distinguish between these two possibilities, we performed Western blot analysis for the presence of D150E and G196D in total membrane preparations and in purified plasma membrane preparations (Leduc-Nadeau et al. 2007). A typical result is presented in Fig. 4, where the top panel shows that the two mutants are similarly expressed in the total membrane preparation (the material obtained from two oocytes was used in each lane). The bottom panel shows that D150E, but not G196D, can be detected in the purified plasma membrane preparation (purified plasma membrane of 40 oocytes was used in each lane). In addition, 24-h preincubation with 5 mM MI significantly stimulated the presence of D150E in the plasma membrane preparation. On average, for four groups of oocytes obtained from different donors, exposure to 5 mM MI for 24 h resulted in a significant ($P < 0.05$) 71 ± 9%

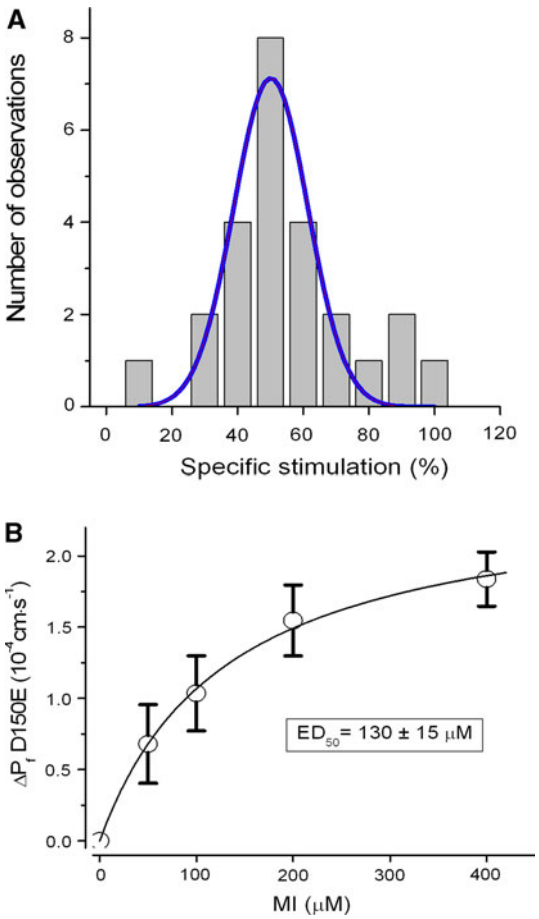

Fig. 3 **a** Distribution of the stimulatory effect of MI on D150E-specific water permeability (P_f). The distribution represents 25 experiments performed using 25 different oocyte donors. Similar to Fig. 1, P_f was measured for three groups of 8–10 oocytes each: noninjected oocytes, D150E-expressing oocytes and D150E-expressing oocytes treated with 5 mM MI for 24 h. The average stimulation in D150E-specific water permeability is 45%, with an SD of 26%. The gaussian curve fitted to the distribution is centered at 50% with a width of 22%. **b** Dose–response curve for the effect of MI on the P_f associated with D150E expression. The unstimulated P_f value of D150E in this series of experiments was 6.3×10^{-4}cm s^{-1}, and only the MI-dependent change in P_f (± SE) is depicted on the graph. The half-maximal effect of MI is reached at 130 ± 15 μM in this series of experiments

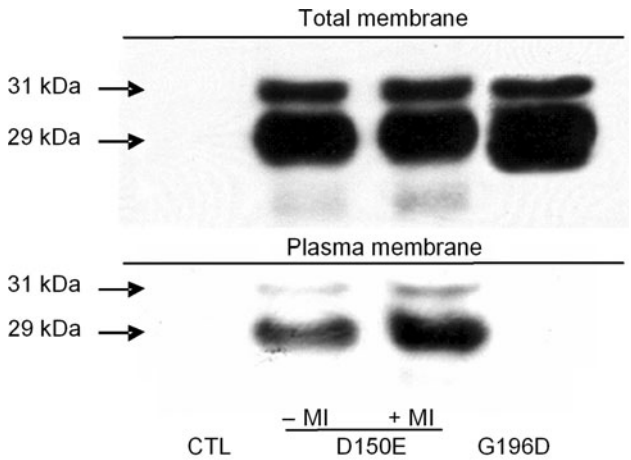

Fig. 4 Western blot analysis of the expression of AQP2 mutants. *Top panel* shows the expression level in total membrane preparations of oocytes expressing D150E and G196D. *Bottom panel* depicts the mutant expression levels observed in purified plasma membrane preparations from the same samples. G196D is totally absent from the plasma membrane, while, in this example, the presence of D150E is shown to increase by 52% when the expressing oocytes were exposed to 5 mM MI for 24 h

increase in D150E-specific P_f, which is paralleled by a significant increase by 41 ± 6% ($P < 0.05$) in the amount of D150E channels found at the plasma membrane.

The specificity of the MI action was also investigated by comparing the effect of MI to the effect of the two closely related isomers: D- and L-chiro-inositol (all used at 0.5 mM). Compared to control conditions (0.5 mM mannitol), only MI was capable of significantly increasing the activity of D150E, while both chiro-inositol isomers failed to do so (Fig. 5a, representative of five different experiments). Similarly, when investigating other known compatible osmolytes such as betaine, taurine and glycerol (all at 0.5 mM, $n = 3$ batches of oocytes) for potential effects on D150E, no specific induction was observed aside from the significant effect of MI (Fig. 5b).

Finally, we tested four other NDI-causing AQP2 mutations to see if other mutants could be stimulated by external MI. Injecting 10 ng mRNA in each case, expression of A70D and V71M was not associated with any significant increase in the oocyte P_f. In contrast, expression of K228E and S265L generated a P_f which reached the level observed with D150E. In this series of experiments, only D150E and S256L were significantly stimulated by 24-h incubation in 5 mM MI ($P < 0.05$ in each case). This observation was repeated three times (i.e., for three different donor frogs) where, on average, MI was shown to stimulate S256L activity by 29 ± 17% (data not shown).

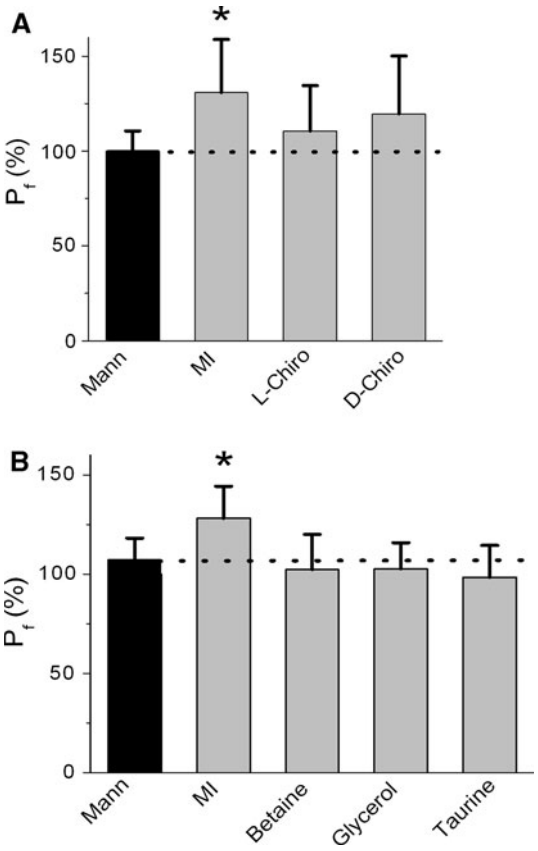

Fig. 5 Specificity of the MI effect on the water permeability (P_f) of oocytes expressing D150E. Groups of 8–10 oocytes were incubated for 24 h in the presence of mannitol or other osmolytes (0.5 mM in each case). In the series of experiments shown in **a**, the effect of MI is compared to the effects of L- and D-chiro-inositol (mean P_f values ± SE, normalized to the mean value measured in the presence of mannitol). In the series of experiments shown in **b**, the effect of MI is compared to the effects of other compatible osmolytes such as betaine, glycerol and taurine (0.5 mM in each case). Only MI gave a significant increase in the D150E-mediated P_f ($P < 0.05$ in **a** and $P < 0.05$ in **b**, $n = 8$ oocytes in each group)

Discussion

The present study considerably improves our understanding of MI's effects in promoting the plasma membrane expression of potentially misfolded proteins. In the two previous reports (Howard et al. 2003; Zhang et al. 2003), a single mutant protein was used (Δ508-CFTR), a single MI concentration was used (in each case) and the experiments were not designed to distinguish between extracellular and intracellular effects of MI. At this point, it is difficult to say if the mechanism of action of MI on misfolded protein expression is different in the present study vs. those of Howard et al. (2003) and Zhang et al. (2003). It is possible that the effect described here using *X. laevis* oocytes comprises part of the effect previously reported in cell cultures. In addition to the cell types used, there are many differences between the three studies. In the case of Howard et al., the fact that the cells were exposed to MI in a hypertonic solution may induce an important effect in itself. Along these lines, we have recently shown that, in a stably transfected cell line (SMIT2-transfected MDCK cells), hyperosmolarity (NaCl or mannitol addition) can efficiently promote the transcription of a vector-contained insert (Bissonnette et al. 2008). This could also have happened to the vector containing Δ508-CFTR in the 3T3 cells (mouse fibroblast cells) used by Howard et al. In addition, when cells are exposed to 300 mM MI for several hours, intracellular MI concentrations may reach a level where the MI can act as a chemical chaperone. This was certainly not the case in the present study since intracellular MI is absent. In the case of Zhang et al. (2003), the intracellular MI concentration reached has not been measured, but even in the presence of putative endogenous Na-coupled MI transporters (SMIT1 and/or SMIT2), it would be surprising if intracellular MI would greatly exceed the value of 30 mM which is found in renal medullary cells and in brain cells (Novak et al. 1999; Fisher et al. 2002). In this case, an effect distinct from the chemical chaperone effect would probably have to be considered.

In the present case, oocytes were used to study the effect of MI on the expression level of wt and mutant aquaporins.

Utilization of oocytes presents two main advantages over most other cell types. First, it allows for a precise determination of the water permeability coefficient through sensitive volumetric measurements. Second, and most importantly, due to their low surface-to-volume ratio and to the fact that their plasma membrane is virtually impermeable to MI, oocytes could be used to demonstrate that the stimulatory effect of 0.5 mM MI on the expression of mutant AQP2 is exclusively an extracellular effect. This effect is also specific since L- and D-chiro-inositol failed to reproduce the effect of MI. It is specific also with respect to lack of substitution by nonrelated compatible osmolytes like betaine, glycerol and taurine. The effect of MI is also specific for certain target mutant proteins. We studied a total of six mutant AQP2s, and only two of them were sensitive to the presence of extracellular MI. These two mutants (D150E and S256L) are characterized by a significant expression level, which increases the oocyte water permeability by at least a factor of 2. In contrast, the mutants A70D, V71M and G196D produce no significant changes in oocyte water permeability. The requirement for a significant basal activity appears to not be sufficient for stimulation by external MI. The case of K228E is an example of a mutant which produces a measurable water permeability without being sensitive to the presence of external MI. Finally, our experiments indicate that the effect of MI on the activity of mutant water channels is mainly through an increase in the density of these channels in the plasma membrane (see Fig. 4) and not due to an increase in the specific activity of the proteins already present.

Unfortunately, as was the case for the studies on the effect of MI on the expression of Δ508-CFTR (Howard et al. 2003; Zhang et al. 2003), the present study cannot provide a mechanism for the effect of MI on AQP2 mutants. Our working hypothesis is that extracellular MI can somehow increase the half-life of some mutant proteins in the plasma membrane. MI could produce this effect by interacting directly with the mutant protein or by interacting with the lipid bilayer in which the protein is inserted. Since MI has no effect on wt AQP2, it is possible that the ubiquitination status of poorly folded protein could be involved in this interaction.

Conclusion

We have found that MI produces a stimulatory effect on the water permeability that is associated with expression of two misfolded forms of the AQP2 water channel. The effect is dose-dependent, compatible with physiological MI concentrations, selective and reproducible. The virtual impermeability of the oocyte membrane to MI has allowed us to show that the effect of MI cannot be due to a chemical chaperone effect, which typically requires a large intracellular concentration to be effective. Our observation may lead to the proposition of a new mechanism by which the external presence of MI may increase the half-life and/or the insertion rate of new mutant AQP2 channels in the plasma membrane.

References

Bagnasco S, Balaban R, Fales HM, Yang YM, Burg M (1986) Predominant osmotically active organic solutes in rat and rabbit renal medullas. J Biol Chem 261:5872–5877

Bissonnette P, Noel J, Coady MJ, Lapointe JY (1999) Functional expression of tagged human Na^+-glucose cotransporter in *Xenopus laevis* oocytes. J Physiol 520 Pt 2:359–371

Bissonnette P, Lahjouji K, Coady M, Lapointe J (2008) Effects of hyperosmolarity on the Na^+-*myo*-inositol cotransporter SMIT2 stably transfected in the Madin-Darby canine kidney cell line. Am J Physiol Cell Physiol 295:C791

Bonifacino J, Weissman A (1998) Ubiquitin and the control of protein fate in the secretory and endocytic pathways 1. Annu Rev Cell Dev Biol 14:19–57

Cheong HI, Cho SJ, Zheng SH, Cho HY, Ha IS, Choi Y (2005) Two novel mutations in the aquaporin 2 gene in a girl with congenital nephrogenic diabetes insipidus. J Korean Med Sci 20:1076–1078

Coady MJ, Wallendorff B, Gagnon DG, Lapointe JY (2002) Identification of a novel Na^+/myo-inositol cotransporter. J Biol Chem 277:35219–35224

Duquette PP, Bissonnette P, Lapointe JY (2001) Local osmotic gradients drive the water flux associated with Na^+/glucose cotransport. Proc Natl Acad Sci USA 98:3796–3801

Fisher SK, Novak JE, Agranoff BW (2002) Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance. J Neurochem 82:736–754

Guyon C, Lussier Y, Bissonnette P, Leduc-Nadeau A, Lonergan M, Arthus MF, Perez RB, Tiulpakov A, Lapointe JY, Bichet DG (2009) Characterization of D150E and G196D aquaporin-2 mutations responsible for nephrogenic diabetes insipidus: importance of a mild phenotype. Am J Physiol Renal Physiol 297:F489–F498

Holub BJ (1986) Metabolism and function of *myo*-inositol and inositol phospholipids. Annu Rev Nutr 6:563–597

Howard M, Fischer H, Roux J, Santos BC, Gullans SR, Yancey PH, Welch WJ (2003) Mammalian osmolytes and *S*-nitrosoglutathione promote ΔF508 cystic fibrosis transmembrane conductance regulator (CFTR) protein maturation and function. J Biol Chem 278:35159–35167

Lapointe JY, Gagnon MP, Gagnon DG, Bissonnette P (2002) Controversy regarding the secondary active water transport hypothesis. Biochem Cell Biol 80:525–533

Leduc-Nadeau A, Lahjouji K, Bissonnette P, Lapointe JY, Bichet DG (2007) Elaboration of a novel technique for purification of plasma membranes from *Xenopus laevis* oocytes. Am J Physiol Cell Physiol 292:C1132–C1136

Loo D, Zeuthen T, Chandy G, Wright E (1996) Cotransport of water by the Na^+/glucose cotransporter. Proc Natl Acad Sci USA 93:13367

Marr N, Bichet DG, Hoefs S, Savelkoul PJ, Konings IB, De Mattia F, Graat MP, Arthus MF, Lonergan M, Fujiwara TM, Knoers NV, Landau D, Balfe WJ, Oksche A, Rosenthal W, Muller D,

Van Os CH, Deen PM (2002) Cell-biologic and functional analyses of five new aquaporin-2 missense mutations that cause recessive nephrogenic diabetes insipidus. J Am Soc Nephrol 13:2267–2277

McDill BW, Li SZ, Kovach PA, Ding L, Chen F (2006) Congenital progressive hydronephrosis (cph) is caused by an S256L mutation in aquaporin-2 that affects its phosphorylation and apical membrane accumulation. Proc Natl Acad Sci USA 103:6952–6957

McLaurin J, Golomb R, Jurewicz A, Antel JP, Fraser PE (2000) Inositol stereoisomers stabilize an oligomeric aggregate of Alzheimer amyloid beta peptide and inhibit abeta-induced toxicity. J Biol Chem 275:18495–18502

Mishra R, Bhat R, Seckler R (2007) Chemical chaperone-mediated protein folding: stabilization of P22 tailspike folding intermediates by glycerol. Biol Chem 388:797–804

Novak JE, Turner RS, Agranoff BW, Fisher SK (1999) Differentiated human NT2-N neurons possess a high intracellular content of *myo*-inositol. J Neurochem 72:1431–1440

Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 313:1137–1140

Schröder M, Kaufman RJ (2005) The mammalian unfolded protein response. Annu Rev Biochem 74:739–789

Tamarappoo BK, Verkman AS (1998) Defective aquaporin-2 trafficking in nephrogenic diabetes insipidus and correction by chemical chaperones. J Clin Invest 101:2257–2267

Welch WJ, Brown CR (1996) Influence of molecular and chemical chaperones on protein folding. Cell Stress Chaperones 1:109–115

Zhang XM, Wang XT, Yue H, Leung SW, Thibodeau PH, Thomas PJ, Guggino SE (2003) Organic solutes rescue the functional defect in delta F508 cystic fibrosis transmembrane conductance regulator. J Biol Chem 278:51232–51242