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THE PERIVASCULAR POOL OF AQUAPORIN-4 MEDIATES THE EFFECT OF OSMOTHERAPY IN POST-ISCHEMIC CEREBRAL EDEMA

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Abstract

Objective—Osmotherapy with hypertonic saline (HS) ameliorates cerebral edema associated with experimental ischemic stroke. We tested the hypothesis that HS exerts its anti-edema effect by promoting an *efflux* of water from brain via the perivascular aquaporin-4 (AQP4) pool. We utilized mice with targeted disruption of the gene encoding α -syntrophin (α -Syn^{-/-}) that lack the perivascular AQP4 pool but retain the endothelial pool of this protein.

Design—Prospective laboratory animal study.

Setting—Research laboratory in a university teaching hospital.

Measurements and Main Results—Halothane-anesthetized adult male wildtype (WT) C57B/6 and α -Syn^{-/-} mice were subjected to 90 min of transient middle cerebral artery occlusion (MCAO) and treated with either a continuous intravenous infusion of 0.9% saline (NS) or 3% HS (1.5 mL/Kg/hr) for 48 hr. In the first series of experiments (n = 59), brain water content analyzed by wet-to-dry ratios in the ischemic hemisphere of WT mice was attenuated after HS (79.9 ± 0.5% mean ± SEM) but not after NS (82.3 ± 1.0%) treatment. In contrast in α -Syn^{-/-} mice, HS had no effect on the postischemic edema (HS: 80.3 ± 0.7% NS: 80.3 ± 0.4%). In the second series of experiments (n = 31), treatment with HS attenuated post-ischemic BBB disruption at 48 hr in WT mice but not in α -Syn^{-/-} mice; α -Syn deletion alone had no effect on BBB integrity. In the third series of experiments (n=34), α -Syn^{-/-} mice treated with either HS or NS had smaller infarct volume as compared with their WT counterparts.

Conclusions—These data demonstrate that: 1) osmotherapy with HS exerts anti-edema effects via the perivascular pool of AQP4 2) HS attenuates BBB disruption depending on the presence of

perivascular AQP4, and 3) deletion of the perivascular pool of AQP4 alleviates tissue damage following stroke, in mice subjected to osmotherapy as well as in non-treated mice.

Keywords

Osmotherapy; stroke; focal cerebral ischemia; reperfusion; infarct; cerebral edema; aquaporins

Limited therapeutic modalities are available for the cerebral edema that develops following stroke and that contributes significantly to the morbidity and mortality of this common condition (1). Osmotherapy remains the cornerstone of medical therapy for cerebral edema from diverse etiologies including ischemic stroke (2–9). Historically, a variety of osmotic agents have been utilized to improve intracranial elastance including urea, mannitol, sorbitol, and glycerol. The accepted tenet is that the potent anti-edema effect of osmotic agents is affected primarily via egress of water from the interstitial and extracellular space into the intravascular compartment through an intact blood-brain barrier (BBB) (2,3,5–10). In addition to this primary action, osmotic agents have been shown to exert beneficial non-osmotic cerebral effects that include augmentation of regional cerebral blood flow (CBF) resulting in enhanced tissue oxygen delivery, free radical scavenging, and modulation of cerebrospinal fluid formation and reabsorption (3–5). In view of their superior toxicity profile, hypertonic saline (HS) solutions are gaining renewed interest and acceptance as osmotherapeutic agents for the treatment of cerebral edema in a variety of brain injury paradigms (2,3,5–10). We have shown previously in well-characterized animal models of ischemic stroke, that institution and maintenance of a hyperosmolar state with continuous intravenous (IV) HS infusion ameliorates cerebral edema and improves survival (6–9).

The mechanisms of edema formation following cerebral ischemia are complex and have not been completely elucidated. Of recent, aquaporin-4 (AQP4), the most abundant water channel in brain, has been implicated in the pathogenesis of cerebral edema in a variety of brain injury paradigms including ischemic stroke (11–21). AQP4 is strongly enriched at the brain-blood interface (22,23). Semiquantitative analyses based on immunogold electron microscopy have revealed two AQP4 pools at this site: a major perivascular pool, localized to the luminal membranes of astrocytic endfeet, and a minor endothelial pool, localized to the luminal as well as abluminal membranes of the endothelial cells (18). The endfoot pool of AQP4 is anchored by the dystrophin associated protein (DAP) complex (24) and is deleted in mdx mice (which lack the DAP complex) and in mice with targeted disruption of the gene encoding α -syntrophin (α -Syn^{-/-} mice). α -Syntrophin is a member of the DAP complex. Both types of mice show a reduced extent of cerebral edema after ischemic stroke (14–16), suggesting that the perivascular pool of AQP4 is rate limiting for water influx during edema formation. These results provide a mechanistic basis for the observation that AQP4^{-/-} mice are partially protected against the development of post-ischemic cerebral edema (12).

So far no studies have investigated whether AQP4 mediates the water flux that is induced by osmotherapy. Utilizing HS as a tool in a well-characterized mouse model of transient focal ischemia, the present study was designed to test the hypotheses that: 1) the perivascular pool of AQP4 is selectively involved in the egress of water from the brain into the intravascular compartment with osmotherapy 2) BBB disruption is not exacerbated with osmotherapy, and 3) deletion of AQP4 from the perivascular membranes alleviates post-ischemic tissue damage in the absence as well as in the presence of osmotherapy.

Material and Methods

General Preparation and Animal Surgery

The experimental protocol was approved by the Institutional Animal Care and Use Committee and conformed to the National Institutes of Health guidelines for the care and use of animals in research. All techniques were conducted as previously described (25) with modifications (26). All experiments were conducted with male mice homozygous for targeted disruption of the gene encoding α -synuclein (α -Syn^{-/-}). WT C57B/6 mice were used as controls. α -Syn^{-/-} mice were bred on a C57B/6 background more than 10 generations to avoid effects of differing genetic strains (27).

Focal Ischemia

WT and α -Syn^{-/-} mice with body weight of 22–32 g were anesthetized with 1% to 1.2% halothane in oxygen-enriched air and rectal temperatures were maintained at $37 \pm 0.5^\circ\text{C}$ with heating lamps during the entire surgical procedure until emergence from anesthesia. With aseptic surgical techniques, the right jugular vein was cannulated, tunneled subcutaneously, exteriorized, and tethered to the skin for vascular access and fluid administration. Rectal temperature was maintained throughout surgical procedures, during ischemia, and until emergence from anesthesia.

Transient focal ischemia (90 min) was produced by middle cerebral artery occlusion (MCAO) (25,26) using an intraluminal suture technique in combination with laser-Doppler flowmetry (LDF) (Moor Instruments Ltd, Model MBF3D, England) over the ipsilateral parietal cortex, as described previously (26). Briefly, a 7-0 nylon monofilament was inserted via an external carotid artery stump to a point 6-mm distal to the internal carotid artery/ pterygopalatine bifurcation, and the common carotid was temporarily occluded with a 6-0 nylon suture. MCAO was verified at 30 min by allowing the animal to emerge from anesthesia and performing a neurological deficit scoring (NDS) as follows: 0 = normal motor function, 1 = flexion of torso and of contralateral forelimb on tail lift, 2 = circling to the contralateral side but normal posture at rest, 3 = leaning to contralateral side at rest, 4 = no spontaneous motor activity. Animals that did not show reduction in LDF signal or that had an NDS ≤ 1 on emergence from anesthesia were excluded from the study. Mice with clear neurological deficits (score ≥ 2) were re-anesthetized for withdrawal of the suture and reperfusion after 90 min of MCAO. Animals were housed in separate cages at room temperature until the end of the experiment. After completion of treatments at 48 hr post-MCAO, the jugular catheter was carefully removed and brains were harvested for assessment of cerebral edema, BBB disruption or assessment of injury volume.

Assessment of Regional Brain Edema

Following treatment for desired durations, mice were killed by decapitation under deep halothane (5%) anesthesia. The brain was quickly removed and dissected along the interhemispheric fissure into the ischemic and non-ischemic cerebral hemispheres. Brain edema was assessed by comparing wet-to-dry ratios (WDR) as described previously (6–9, 28). Tissues were weighed with a scale to within 0.1 mg. Dry weight of the brain was determined after heating the tissue for 3 days at 100°C in a drying oven. Tissue water content was then calculated as $\% \text{ H}_2\text{O} = (1 - \text{dry wt/wet wt}) \times 100\%$ (28).

Assessment of BBB Integrity

BBB permeability was assessed by the Evans blue (EB) extravasation method (29) with modifications (7). Briefly, EB (2% in 0.9% saline; 3 ml/kg) was administered intravenously 3 hr prior to sacrifice. Via a thoracotomy under halothane anesthesia, intracardiac perfusion was

then performed through the left ventricle with saline to remove intravascular EB dye, and continued until the fluid from the right atrium became colorless. Mice were then decapitated, brains quickly removed, and dissected into right (ischemic) and left (non-ischemic) hemispheres. Each hemisphere was weighed and homogenized in 4 ml of 50% trichloroacetic acid solution. After centrifugation at 10,000 g for 30 min, the supernatants were diluted with ethanol (1:3), and EB concentration was determined with a spectrophotometer at 620 nm for absorbance against a standard curve. EB extravasation was expressed as the ratio of absorbance intensity in the ischemic hemisphere to that in the non-ischemic hemisphere (EB extravasation index) as described previously (7).

Assessment of infarct volume

The forebrain was sliced into five 2-mm thick coronal sections, which were stained with 1% triphenyltetrazolium chloride (TTC), as described previously (25,26). Infarct volume was measured with digital imaging. The infarcted area was numerically integrated across each section and over the entire ipsilateral ischemic hemisphere. Infarct volumes were numerically integrated across each section and over the ipsilateral hemisphere. Infarct volumes were measured separately in cerebral cortex and the caudoputamen (CP) complex and expressed in mm³, as previously described (25,26).

Assessment of plasma osmolality

At the end of the experiment, a sample of blood was drawn by cardiac aspiration to determine serum osmolality (mOsm/L) with an automated freezing point depression micro-osmometer (Advanced Instruments, Inc., Norwood, MA) (6,7,9).

Experimental Groups

In the first series of experiments (n = 46), WT and α -Syn^{-/-} mice subjected to MCAO were randomized to receive either continuous IV infusion of 0.03 mL/hr ($\approx$ 1.5 mL/kg/hr) of 0.9% saline (NS; 308 mOsm/L) or 3% HS (1023 mOsm/L). Naïve WT and α -Syn^{-/-} mice served as controls (n = 4 each). HS was instituted as a mixture of acetate:chloride (50:50; pH = 6.5–7.0) to avoid hyperchloremic acidosis. Treatments were started at the onset of reperfusion and continued for 48 hr. NDS and daily weights were documented and total body weight loss was analyzed at each time point. Brain water content in the ischemic and non-ischemic hemisphere was analyzed by WDR at the end of the experiment.

In the second series of experiments, WT and α -Syn^{-/-} mice (n = 32) subjected to MCAO were randomized to receive continuous IV infusion of 1.5 mL/Kg/hr of NS or 3% HS for 48 hr. BBB integrity was assessed by EB extravasation method at the end of the experiment. Sham-operated WT and α -Syn^{-/-} mice that had all surgical procedures (neck incision, instrumentation and vascular catheterization) except for MCAO served as controls (n=5 each).

In the third series of experiments, WT and α -Syn^{-/-} mice (n = 34) subjected to MCAO were randomized to receive continuous IV infusion of 1.5 mL/Kg/hr of NS or 3% HS for 48 hr. Infarct volume was analyzed by TTC staining (corrected for brain swelling) at the end of the experiment.

In a separate set of experiments we determined baseline serum osmolality in naïve WT and α -syn^{-/-} mice (n= 5 each).

Statistical Analysis

All values are expressed as mean $\pm$ SEM. Physiologic parameters, plasma osmolality and mean LDF measurements, differences in hemispheric water content, and EB extravasation among treatment groups were determined by one-way analysis of variance with post hoc Newman

Keuls test. Neurologic deficit score is presented as median (with 25% and 75% quartiles) and analyzed by the non-parametric Mann-Whitney *U* test. The criterion for statistical significance was $P < 0.05$.

Results

In the first series of experiments, 4 mice were excluded from the final analysis because of dislocation of the venous catheter. Additionally, 5 mice did not meet the criterion for reduction in LDF-signal for successful MCAO. Physiologic parameters including rectal temperature and serum glucose were within normal range in all experimental groups. There were no differences between control and experimental groups in mortality rates prior to the desired experimental end point (48 hr post-MCAO) (Table 1). Plasma osmolality was significantly elevated in WT and α -syn^{-/-} mice treated with 3% HS as compared to their counterparts treated with NS. We did not discern any differences in mortality rates % weight loss with 3% HS as compared to WT mice treated with NS. Surgical sham α -syn^{-/-} mice had significantly lower water content in both hemispheres ($76.7 \pm 0.1\%$; $76.9 \pm 0.3\%$) than WT ($78.1 \pm 0.3\%$; $78.1 \pm 0.2\%$) mice (Figure 1). In WT and α -syn^{-/-} mice treated with NS, MCAO led to an increase in brain water content in the ipsilateral hemisphere. At 48 hr of reperfusion – when edema is fully developed – this increase was significantly more pronounced in WT mice than in α -syn^{-/-} mice, confirming the protective effect of removing perivascular AQP4 (18). Treatment of WT mice with HS attenuated the ischemia-evoked increase in water content at 48 hr of reperfusion. This effect was evident in the ipsi- as well as in the contralateral hemisphere. Treatment with HS had no effect on the brain water content in α -syn^{-/-} mice (Figure 1). For all comparisons, mice that had received NS instead of HS were used as a reference group.

The second series of experiments aimed at investigating the effect of osmotherapy on the integrity of the BBB, in the absence and presence of perivascular AQP4. One mouse in the α -syn^{-/-}-HS group was excluded because of premature dislocation of the venous catheter. The LDF-signal during MCAO and physiologic parameters and mortality rates were similar in all experimental groups (Table 2). Neurological deficit scores and total body weight loss were also similar among different treatment groups. At the end of the experiment, plasma osmolality was significantly elevated in WT and α -syn^{-/-} mice treated with HS as compared to their counterparts treated with NS. Following sham operations, the Evans Blue Extravasation Index was 0.99 ± 0.07 in WT and 1.04 ± 0.02 in α -syn^{-/-} mice (not significantly different). BBB disruption was similar in WT and α -syn^{-/-} treated with NS at 48 hr of reperfusion (Figure 2). Treatment with HS attenuated post-ischemic BBB disruption in WT mice but not in α -syn^{-/-} mice.

The third series of experiments aimed at resolving the effect of α -syn gene deletion on infarct volume, alone or in combination with osmotherapy. Three mice were excluded from the final analysis because of dislocation of the venous catheter and additional two mice did not meet the criterion for reduction in LDF-signal for successful MCAO. LDF-signal during MCAO, physiologic parameters and mortality rate were similar in all experimental groups (Table 3). The neurological deficit scores and total body weight loss were similar among different treatment groups. At the end of the experiment, plasma osmolality was significantly elevated in WT and α -syn^{-/-} mice treated with HS as compared to their counterparts treated with NS. In α -syn^{-/-} mice, infarct volume at 48 hr of reperfusion was significantly attenuated in the cortex, CP, and total hemisphere as compared to their WT counterparts (Figure 3 and 4). Replacing NS with HS did not significantly affect infarct volume in WT or α -syn^{-/-} mice.

Discussion

The water channel AQP4 is enriched in perivascular astrocyte membranes where it is densely packed in distinctive rafts called orthogonal arrays of proteins (24). Judged by its peculiar mode of expression AQP4 is likely to be one of the most abundant molecules at the brain-blood interface and has been proposed to play a major role in edema associated with a variety of brain injury paradigms (11–21). The functional role of the various pools of AQP4 is presently under investigation. By drawing advantage of a tailor made transgenic model, the present study provides new insight in the physiological and pathophysiological roles of perivascular AQP4. First, we show that this AQP4 pool mediates the anti-edema effect of osmotherapy with HS in a well-characterized model of ischemic stroke. Second, HS attenuates BBB disruption depending on the integrity of the perivascular AQP4 pool. Third, deletion of the perivascular pool of AQP4 alleviates post-ischemic tissue damage, with or without the administration of osmotherapy. These data provide important insights into the mechanisms underlying the beneficial effect of osmotherapy following ischemic stroke and support the idea that the perivascular pool of AQP4 might be a promising target for therapy aiming at ameliorating post-ischemic edema.

Although HS solutions were first investigated experimentally over 85 years ago (30) and subsequently utilized clinically for small volume resuscitation in patients with shock (31), of recent these solutions have received renewed attention as hyperosmolar agents and are being increasingly utilized clinically to alleviate cerebral edema in a variety of brain injury paradigms (2,3,5). Because sodium chloride (reflection coefficient = 1.0) (2–4,10) is completely excluded from brain regions with an intact BBB, it has been proposed that HS may be a more favorable osmotic agent than the conventional osmotic agent mannitol (reflection coefficient = 0.9) which has been the mainstay in clinical practice since 1960. We have previously demonstrated that induction of a hyperosmolar state with HS effectively attenuates ischemia-evoked cerebral edema without accentuating ischemia-evoked BBB breakdown (6). In addition to other beneficial non-osmotic properties of HS solutions (5,10), its anti-inflammatory action leading to less BBB breakdown has also been proposed to afford benefit (31). Furthermore, HS may be a more desirable agent for maintaining a “euvolemic hyperosmolar” state in a variety of brain injury paradigms as opposed to the conventional osmotic agent, mannitol (5,10).

In the present study, we used HS as a tool and explored if its osmotic action depends on the integrity of the perivascular pool of AQP4. Our hypothesis was that the latter AQP4 pool mediates the egress of water that is induced when the brain is exposed to HS. In preliminary experiments, we determined that brain edema is maximal at 48 hr in our model of transient focal ischemia (data not shown). As in our previous studies (6–9), therapy with HS was used as a continuous infusion (1.5 mL/kg/hr) to maintain a euvolemic hyperosmolar state and to maintain a constant osmotic gradient sufficient to cause egress of water from the injured and non injured brain. This rate of infusion is commensurate with maintenance fluid requirement in the human (9). Furthermore, we used HS as chloride: acetate mixture to avoid hyperchloremic acidosis that occurs with use of concentrated chloride solutions alone. As in our previous studies (6–9), wet-to dry ratios comparisons were used as a simple and reproducible assessment of brain water in both ischemic and nonischemic hemispheres. A major conclusion of this study is that HS makes a difference (compared with NS) only in those situations where perivascular AQP4 is available for water egress. When perivascular AQP4 is lacking—because of postischemic down-regulation (32) or following α -syn deletion—the beneficial effect of HS vs. NS is negated. The findings are thus consistent with our hypothesis that perivascular AQP4 mediates the egress of water induced by exposure to HS.

We observed less brain water content in naïve α -syn^{-/-} mice as compared with their WT counterparts. The explanation for this dataset remains unclear at present as parallel experiments failed to reveal differences in the baseline level of serum osmolality.

There is a distinct possibility that sustained hypertonicity upregulates a variety of proteins including AQP4. We have recently demonstrated that total AQP4 protein expression is upregulated in the ischemic hemisphere in animals treated with HS as compared with normal saline-treated animals in a well-characterized rat model of permanent focal ischemia (8). α -Syn deletion also caused a modest reduction in perivascular Kir4.1 in our previous study (16). However, other molecules engaged in transport processes across the blood-brain interface (e.g., monocarboxylate transporter 1, glucose transporter 1, excitatory amino acid transporter 2, and the NaKCl₂ cotransporter), were not affected in this study.

The mechanisms of cerebral edema following focal ischemia are complex and not completely elucidated. Historically, postischemic edema has been divided into a cytotoxic component secondary to energy failure and a delayed vasogenic component secondary to BBB breakdown with leakage of plasma constituents (33). Other secondary mechanisms that have been shown to play a significant role in accentuating ischemia-evoked cerebral edema include impedance of cerebral venous return from cerebral swelling, intrahemispheric diaschisis (34), inflammation accentuating BBB disruption (35,36), neurohormonal responses (37), and induction of growth factors (38). Of recent, AQP4—the most abundant of the aquaporin water channels in brain—has been implicated in the pathogenesis of cerebral edema in a variety of brain injury paradigms including ischemic stroke (11,12,14,15,18,21). AQP4 has been shown to facilitate resorption of excess fluid in vasogenic cerebral edema associated with brain tumor and contusive injury (20) and bacterial abscess (19). However, the specific role and function of the perivascular AQP4 pool remains to be fully elucidated. Analyses of gene-targeted animals that lack perivascular AQP4 have indicated that this AQP4 pool is rate limiting for the rapid water exchange that occurs between the blood and brain in the accumulation and resolution phases of brain edema (14–18). Specifically, a selective deletion of the perivascular AQP4 pool by targeted disruption of α -syn causes a pronounced decrease in the extent of brain edema after a transient ischemic insult (14). α -Syn contributes to the organization of the dystrophin complex in astrocytes (39) and is essential for anchoring of AQP4 to the perivascular endfoot membranes (24).

In keeping with our previous studies (6–9), regional brain water content was attenuated by 1% to 2% in both the ischemic and nonischemic hemispheres with HS treatment. This magnitude of reduction in water content translates to >90 mL reduction in human brain volume (a geometric function of water content) (4,40). The translational significance of these findings is important, as reduction in water content of this magnitude can be life-saving for patients with poor intracranial elastance resulting from large hemispheric strokes. As noted above, no such effect was presently observed in α -syn^{-/-} mice, indicating that the egress of water from the brain is mediated by the perivascular pool of AQP4. This finding leaves us with a new concept as to the mechanism of osmotherapy: the osmotic agents act by setting up an osmotic gradient that drives water through a rate limiting pool of perivascular AQP4. This concept is consistent with the bidirectionality of water flux through AQP4 (15). Using *in vivo* multiphoton imaging we have recently demonstrated (41) that hyponatremic edema causes a volume increase of astrocytes that are in close proximity to brain microvessels. These data confirm that astrocytes are sites of water entry and underline the importance of cellular (cytotoxic) edema in the buildup phase of brain edema. Our *in vivo* imaging data thus support the concept that the perivascular AQP4 pool mediates exchange of water across the brain-blood interface.

AQP4 is also expressed in luminal and abluminal endothelial cell membranes, albeit in low amounts compared with the expression level in perivascular endfeet (18). The endothelial pool

of AQP4 is unchanged after targeted disruption of the α -syn^{-/-} gene (18). For water to enter the astrocyte compartment in the brain it has to pass from the capillary lumen through three plasma membranes (luminal endothelial, abluminal endothelial, and luminal perivascular) of which the latter normally contains the highest density of AQP4 water channels. However, when reduced by >90% following α -syn deletion (18) the perivascular AQP4 pool seems to become rate limiting for water exchange—in either direction—at the blood brain interface. Passage of water in the narrow cleft between the astrocyte endfeet may be impeded in the α -syn^{-/-} mice because of the slight swelling of these endfeet (15,17).

It needs to be emphasized that the water flux across the brain-blood interface is not mediated exclusively by AQP4. In addition to a slow diffusion through the plasma membrane, water is subjected to co-transport with ions and organic molecules. Such co-transport may occur, e.g., through monocarboxylate, glucose, and potassium/chloride transporters. We have examined the known water transporting molecules at the brain-blood interface and none of these (except perivascular AQP4) seems to be affected by disruption of the α -syn gene (18). Thus, our data strongly suggest that the effects presently observed after α -syn deletion can be attributed to the loss of perivascular AQP4.

Our study has limitations. Rectal temperature was maintained at 37°C ± 0.5°C in all animals during surgical procedures by placing them on a warming blanket. Although we did not measure brain temperature in our experiments, previous work in our laboratory and by others have demonstrated that maintenance of body temperature at 37°C ± 0.5°C achieves physiologic levels of brain temperature. We did not measure and track serial serum sodium levels in our experimental paradigm. Although the murine model has several advantages because of inclusion of transgenic strain, it has a small blood volume that precludes these measures. Therefore, we opted to determine serum osmolality rather than serum sodium at the end of the experiment (48 hr postischemia). It is well known that tight glycemic control is critical for stroke outcome. Although our data demonstrates a wide variation in serum glucose, it is to be noted that there were no statistical differences in any of these values (48 hr postischemia). Furthermore, our data does not support that osmotherapy had any effect on serum glucose levels. We used young adult male animals in our study. On the average α -syn^{-/-} mice were 3 months older than their WT counterparts in our study to ensure that animals were weight-matched. Body weight is the critical determinant of consistency of injury volume in our model of ischemic stroke because vascular anatomy varies with body weight. Our study cannot comment on the effect of age on the functional role of various domains of AQP4 following cerebral ischemia.

In the present study we did not observe robust improvements as in our previous studies in the rat (6,7) in functional neurologic deficits with HS, although mortality was attenuated as compared with treatment with NS. Species differences and large injury volume with 90-min MCAO may account for the lack of robust differences in functional outcome. Nevertheless, the primary goal of this study was not to demonstrate efficacy of HS as in our previous studies, but to use it as a tool in understanding the mechanism of efflux of water from brain during osmotherapy. Our conclusions are also tempered by the possibility that the α -syn deletion could have affected the integrity of the BBB and that this could explain why the effect of HS was abrogated in the α -syn^{-/-} animals. Our data suggest that the BBB remains intact following α -syn deletion, in keeping with our previous studies (17). However, a more detailed evaluation of BBB characteristics following α -syn deletion is warranted. Interestingly, after MCAO, treatment with HS significantly restored BBB function in WT mice but had no significant effect in α -syn^{-/-} mice. Infarct volume was significantly attenuated in α -syn^{-/-} mice compared with WT as demonstrated previously (15,17). The explanation for this observation remains elusive at present, but it is likely that it reflects an alleviation of the secondary effects of edema.

Conclusions

To our knowledge, this is the first study to investigate the effect of HS in a well-characterized murine model of ischemic stroke. We have shown that the antiedema effect of HS depends on the integrity of the perivascular pool of the AQP4 water channels. This is in line with the bidirectionality of water flux through AQP4 (15,19). In other words, the same AQP4 pool that mediates water influx during edema formation seems to represent a major route of water efflux in osmotherapy. We also conclude that removal of perivascular AQP4 by α -syn deletion does not seem to alter BBB integrity. The perivascular AQP4 pool is a potential target for the treatment of ischemia-evoked cerebral edema.

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Figure 1.

% Brain water content in wild type (WT) and α -Syn^{-/-} surgical shams (n = 4 each) (in each hemisphere) and the ipsilateral ischemic (I) and contralateral (C) nonischemic hemisphere of WT and α -Syn^{-/-} mice treated with 0.9% saline (NS) or hypersaline saline (HS) for 48 hrs following 90-min middle cerebral artery occlusion (WT-NS: n = 12; WT-HS: n = 12; α -Syn^{-/-}-NS: n = 6; α -Syn^{-/-} HS: n = 6). **p* < 0.05 vs. corresponding ipsilateral ischemic and contralateral nonischemic hemisphere.

Figure 2.

Blood brain barrier disruption as estimated by Evans blue (EB) extravasation, expressed as the ratio of absorbance intensity in the ischemic hemisphere to that in the nonischemic hemisphere (EB extravasation index) in various treatment groups at 48 hrs following middle cerebral artery occlusion (n = 5 each). * $p < 0.05$ vs. wild type-0.9% saline (WT-NS). HS, hypertonic saline.

Figure 3.

Triphenyltetrazolium chloride-determined infarct volume (mm³) in the cerebral cortex, caudoputamen complex, and the ipsilateral hemisphere at 48 hrs post-middle cerebral artery occlusion in various treatment groups (wild type-0.9% saline [WT-NS]: n = 5; WT-hypertonic saline [HS]: n = 5; α-Syn^{-/-}-NS: n = 6; α-Syn^{-/-}-HS: n = 6). **p* < 0.05 vs. WT-NS; †*p* < 0.05 vs. WT-HS. CP, caudoputamen.

Table 1

Summary of physiological variables and NDS in mice used for experiments on brain water content at 48 hr of reperfusion following 90 min MCAO

	WT-NS	WT-HS	α -Syn ^{-/-} -NS	α -Syn ^{-/-} -HS
N (24 hr reperfusion)	12	12	6	6
N (48 hr reperfusion)	12	12	6	6
Reduction in LDF-signal from baseline (%) during MCAO	84 ± 2	85 ± 2	85 ± 4	90 ± 2
Rectal Temperature (°C)				
Pre-ischemia	36.7 ± 0.2	36.2 ± 0.1	36.0 ± 0.1	36.1 ± 0.2
MCAO	36.6 ± 0.1	36.7 ± 0.2	36.8 ± 0.1	37.0 ± 0.1
Immediate Reperfusion	36.0 ± 0.2	36.5 ± 0.1	36.5 ± 0.1	36.3 ± 0.2
Body weight (g)				
Day 1 (pre-ischemia)	22.0 ± 0.3	21.3 ± 0.2	29.3 ± 0.8	29.3 ± 0.7
Day 2	19.8 ± 0.3	19.6 ± 0.2	26.3 ± 0.8	26.5 ± 0.7
Day 3	17.5 ± 0.4	17.4 ± 0.2	24.0 ± 0.6	24.0 ± 0.7
Weight loss (%)	20.5 ± 1.4	18.3 ± 0.8	18.2 ± 0.4	18.2 ± 1.2
Blood Glucose (mg/dL) NDS	188 ± 23	148 ± 18	140 ± 28	143 ± 22
Day 1 (pre-ischemia)	2 (2–2)	2 (2–2)	2 (2–2)	2 (2–2)
Day 2	1 (1–2.5)	2 (1–2.5)	1 (1–2)	1 (1–2)
Day 3	1 (1–2.5)	2 (1–3)	1 (1–2)	1 (1–2)
Plasma Osmolality (mOsm/L) at end of experiment	328 ± 2	345 ± 5 [*]	322 ± 3	350 ± 5 [†]
Mortality (died/total mice utilized)	3/15	0/12	1/7	2/8

NS = 0.9% saline; HS = 3% hypertonic saline; LDF = Laser Doppler Flowmetry Values are mean ± SEM;

^{*} $p < 0.05$ vs WT-NS;

[†] $p < 0.05$ vs α -syn^{-/-}-NS

Table 2

Summary of physiologic variables and NDS in mice used for experiments on BBB disruption at 48 hr of reperfusion following 90 min MCAO

	WT-NS	WT-HS	α -syn ^{-/-} -NS	α -syn ^{-/-} -HS
N	5	5	5	5
Reduction in LDF-signal from baseline (%) during MCAO	87 ± 4	93 ± 2	93 ± 2	84 ± 4
Rectal Temperature (°C)				
Pre-ischemia (Baseline)	35.9 ± 0.1	35.8 ± 0.2	36.2 ± 0.1	35.8 ± 0.1
MCAO	36.5 ± 0.1	36.2 ± 0.1	36.6 ± 0.1	36.6 ± 0.2
Immediate Reperfusion	36.3 ± 0.1	36.6 ± 0.1	36.5 ± 0.1	36.5 ± 0.1
Body weight (g)				
Day 1 (pre-ischemia)	26.6 ± 1.1	22.6 ± 1.6	31.8 ± 0.2	31.4 ± 1.0
Day 2	24.8 ± 1.0	20.6 ± 1.8	29.8 ± 0.2	29.2 ± 0.9
Day 3	23.0 ± 0.8	19.0 ± 2.0	27.4 ± 0.2	27.0 ± 0.8
Total weight loss (%)	13.4 ± 1.2	16.8 ± 3.2	13.8 ± 0.7	14.0 ± 1.0
Blood Glucose (mg/dL) NDS	116 ± 18	122 ± 28	160 ± 19	133 ± 15
Day 1	2 (1.75-2.25)	2 (2-2)	2 (2-2)	2 (2-2)
Day 2	1 (1-2)	2 (2-2.25)	2 (1-2)	2 (1.75-3)
Day 3	1 (1-2)	2 (2-2.25)	2 (1-2)	2 (1-3)
Plasma Osmolality (mOsm/L)	322 ± 3	348 ± 2 [*]	320 ± 5	355 ± 5 [†]
Mortality (died/total mice utilized)	0/5	0/5	1/6	0/5

NS = 0.9% saline; HS = 3% hypertonic saline; LDF = Laser Doppler Flowmetry Values are mean ± SEM;

^{*} $p < 0.05$ vs WT-NS;

[†] $p < 0.05$ vs α -syn^{-/-}-NS

Table 3

Table of physiologic variables and NDS in mice used for evaluation of infarct volume at 48 hr of reperfusion following 90 min MCAO

	WT-NS	WT-HS	α -syn ^{-/-} -NS	α -syn ^{-/-} -HS
N	5	5	6	6
Reduction in LDF-signal from baseline (%) during MCAO	94 ± 2	94 ± 2	93 ± 3	88 ± 3
Rectal Temperature (°C)				
Pre-ischemia (Baseline)	35.5 ± 0.2	36.0 ± 0.1	36.2 ± 0.1	36.2 ± 0.2
MCAO	36.5 ± 0.2	36.5 ± 0.2	37.0 ± 0.1	36.7 ± 0.2
Immediate Reperfusion	36.6 ± 0.1	36.5 ± 0.1	36.7 ± 0.1	36.5 ± 0.1
Body weight (g)				
Day 1 (pre-ischemia)	22.0 ± 0.8	21.8 ± 0.5	29.0 ± 0.7	31.2 ± 0.9
Day 2	20.6 ± 0.7	20.6 ± 0.4	26.8 ± 0.8	28.8 ± 0.9
Day 3	19.6 ± 0.7	19.0 ± 0.5	25.0 ± 0.8	26.7 ± 0.8
Total weight loss (%)	10.0 ± 1.0	12.8 ± 1.6	13.9 ± 1.1	14.4 ± 0.5
Blood glucose (mg/dL) NDS	169 ± 29	145 ± 15	186 ± 38	146 ± 16
Day 1 (pre-ischemia)	2 (2–2)	2 (2–2)	2 (2–2)	2 (2–2)
Day 2	2 (1.75–2.25)	2 (2–3)	1 (1–2)	1 (1–1)
Day 3	2 (1–2.25)	2 (2–3)	1 (1–2)	1 (1–1)
Plasma Osmolality (mOsm/L)	322 ± 1	350 ± 4 [*]	320 ± 5	359 ± 7 [†]
Mortality (died/total mice utilized)	1/6	1/6	2/8	3/9

NS = 0.9% saline; HS = 3% hypertonic saline; LDF = Laser Doppler Flowmetry Values are mean ± SEM;

^{*} $p < 0.05$ vs WT-NS;

[†] $p < 0.05$ vs α -syn^{-/-}-NS