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Dance Round

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

WATER CHANNEL PROTEINS OF ADIPOCYTES

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After the first water channel protein was discovered in the red blood cell membrane in 1985, more than 200 water channel proteins have been identified in all living organisms. In mammalians there are 13 members of the water channel protein family, with two groups of members: aquaporins, which are considered as specific water channels, and aquaglyceroporins, which are permeable to water (however to varying degrees), but are also permeated by other small molecules, in particular glycerol. Adipocytes express aquaporin 1 (AQP1) and aquaglyceroporin (AQP7). Aquaporin 1 is abundant in adipocytes but its functional significance is not yet precisely known. Aquaporin 7 may prevent the acute rise in intracellular osmotic pressure due to glycerol production during lipolysis, which could damage the cell. Dysregulation of AQP7 is increasingly implicated in the pathogenesis of obesity. This is supported by studies on cultured adipocytes and on animals in which AQP7 was knocked down. Such studies are briefly reviewed, and adipopharmacology of AQP7 in obesity is outlined. ***Biomed Rev 2006; 17: 105-110.***

Key words: adipopharmacology**,** aquaporin, aquaglyceroporin, glycerol transport, obesity, water transport

INTRODUCTION

Water channels or water channel proteins are transmembrane proteins that have as their main function the transport of water (1). The first water channel proteins, called today aquaporin 1 (AQP1), was discovered by our group in 1985 in Cluj-Napoca, Romania, reported in publications in 1986 (2,3) and reviewed in subsequent years (1,4-16). The recognition of the priority of Benga's group is growing as can bee seen at www.ad-astra.ro/benga.

Since 1993 more than 200 water channel proteins have been discovered in all living organisms, from bacteria to plants, animals and humans. It was realized that water channel proteins form a large superfamily of proteins (1), with two groups of members: *aquaporins*, which are considered as specific water channels and *aquaglyceroporins*, which are permeable to water (however to varying degrees), but are also permeated by other small molecules, in particular glycerol.

In mammalians there are 13 members of the water channel

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protein family (15). Aquaporins are: AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8. AQP6 and AQP8 are in this group on the basis of sequence analysis, although AQP6 is permeable to anions and AQP8 is permeable to water and urea. Aquaglyceroporins are: AQP3, AQP7, AQP9 and AQP10. Aquaglyceroporins have been suggested to play a role in the regulation of metabolism through regulated glycerol transport (reviewed in 17).

In 1997 a protein belonging to the water channel protein family has been discovered in the adipose tissue and was tentatively named aquaporin-adipose (AQP-ap) (18). Later it was found to be the adipose-specific glycerol channel, a human homologue of AQP7 (19,20). It is agreed nowadays that adipose tissue expresses two water channel proteins: aquaporin 1 (AQP1) and aquaglyceroporin 7 (AQP7). The Table presents an overview of water channel proteins.

In this *Dance Round* the functional significance and some pathological and pharmacological implications of water channel proteins in adipocytes are briefly reviewed.

PHYSIOLOGICAL SIGNIFICANCE OF WATER CHANNEL PROTEINS IN ADIPOCYTES

Although AQP1 is abundant in adipocytes its functional significance is not yet precisely known. In contrast, many studies have been performed regarding these aspects as far as AQP7 is concerned. AQP7 is highly expressed in white adipose tissue, brown adipose tissue and testis (19). Since AQP7 is the sole aquaglyceroporin in the adipose tissue it was hypothesized (20) that AQP7 may function as a glycerol channel in adipocytes and such a role is important considering the metabolic processes in which adipocytes are involved.

Adipose tissue hydrolyze or synthesize triglycerides (TG) in response to the whole body energy balance (21). In case of starvation, adrenalin stimulates hormone sensitive lipase, which hydrolizes TG to free fatty acids (FFA) and glycerol. These compounds released by lipolysis from visceral adipose tissue are delivered to the liver *via* the portal vein, where FFA are re-esterified to TG and incorporated into lipoproteins. Glycerol is used as a substrate for gluconeogenesis in organs expressing glycerokinase, such as liver and kidney, to maintain plasma level of glucose. The rapid increase in glycerol production during lipolysis results in acute rise in intracellular osmotic pressure, which could damage the cell (21); hence, a possible function of AQP7 as a glycerol channel appears of great importance.

REGULATION OF AQP7 EXPRESSION IN ADIPOCYTES

In agreement with such a hypothesis on the function of AQP7 as a glycerol channel were the results of $mRNA^{AQP7}$ levels during fasting and re-feeding in animals. In mice, fasting was associated with increased $mRNA^{AQP7}$ level while re-feeding reduced the level. Plasma glycerol levels also changed in parallel with $mRNA^{AQP7}$ levels (18). In addition, it was found that during the differentiation of 3T3-L1 adipocytes, the amount of glycerol released into the media increased in parallel with augmentation of $mRNA^{AQP7}$ level (21).

Sequences in AQP7 promoter were identified, suggesting interesting relationships in the regulation of AQP7 expression. Such a sequence is the peroxisome proliferator response element (PPRE) site, which binds a heterodimer of peroxisome proliferator-activated receptor gamma (PPARγ) and retinoic acid X receptor alpha (RXRα). The nuclear transcription factor PPARγ controls many adipose-specific genes. As a result of binding of PPARγ and RXRα to PPRE site of AQP7 promoter $mRNA^{AQP7}$ up-regulation occurs in adipocytes. Furthermore, transfection of PPAR/RXR and their ligands stimulated the promoter activity (22). In contrast, insulin down-regulates $mRNA^{AQP7}$ expression. This was documented by experiments on streptozotocin-induced insulin deficient mice, their adipose AQP7 expression being up-regulated (23), whereas treatment of 3T3-L1 adipocytes with insulin dose-dependently suppressed AQP7 expression. Many genes negatively controlled by insulin (such as glucose-6-phosphatase) have a negative insulin response element in their promoter; it was also found in the promoter of AQP7 (23).

To further clarify the physiological function of AQP7 *in vivo*, AQP7 knockout (KO) mice were generated and their phenotype was analyzed (24). No difference was found in plasma FFA levels between wild type (WT) and KO mice, but plasma glycerol levels were significantly lower in fasting KO mice compared with fasting WT mice. When the lipolysis was enhanced by treatment of mice with a β3-adrenergic agonist, plasma FFA levels were increased in KO mice, but the elevation of plasma glycerol was impaired. To test the KO mice tolerance against fasting the animals were subjected to prolonged fasting. This caused severe hypoglycemia in the KO mice with impaired elevation of glycerol level. In another *in vitro* experiment AQP7 was knocked down in 3T3-L1 adipocytes using RNAi. The addition of adrenaline to the culture medium did not change FFA release but significantly impaired glycerol release in AQP7 knockdown cells (25). These results

Table. *Water channel proteins with known physiological implications in man (from Ref 25)*

Water channel protein	Permeability Characteristics	Tissue distribution	Functions
AQP0	Water (low)	Lens fiber cells	Maintaining the transparency and integrity of the lens
AQP1	Water (high)	Red blood cell Kidney proximal tubule Eye: ciliary epithelium Brain: choroid plexus Lung: alveolar epithelial cells	Multiple functions Concentration of urine Production of aqueous humor Production of cerebrospinal fluid Alveolar hydration state
AQP2	Water (high)	Kidney: collecting ducts	Mediates antidiuretic hormone activity
AQP3	Water (high,), glycerol (high), urea (moderate)	Kidney: collecting ducts Respiratory tract: bronchial epithelium, epithelial cells Skin Eye Colon	Reabsorbtion of water into blood Tracheal and bronchial fluid secretion Hydration of skin
AQP4	Water (high)	Kidney: collecting ducts Brain: ependymal cells Lung: bronchial epithelium	Reabsorbtion of water CSF fluid balance Bronchial fluid secretion
AQP5	Water (high)	Salivary glands Lacrimal gland Sweat gland Lung, cornea	Production of saliva Production of tears Production of sweat
AQP6	Water (low), anions ($NO^{3-} > Cl^-$)	Kidney	No definite function found
AQP7	Water (high), glycerol (high), urea (high), arsenite	Adipose tissue, kidney, testis	Transport of glycerol out of adipocytes
AQP8	Water (high)	Testis, kidney, liver, pancreas, small intestine, colon	No definite function found
AQP9	Water (high), glycerol (high), urea (high), arsenite	Liver, leukocytes, brain, testis	No definite function found
AQP10	Water (low), glycerol (high), urea (high)	Small intestine	No definite function found
AQP11	Water	Testis, kidney proximal tubules, liver	Water movement across intracellular membranes

suggest that AQP7 serves as a glycerol channel and is essential for glucose homeostasis against starvation (21, 25).

In order to further clarify the significance of AQP7 the mutations in human AQP7 gene were studied. Sixty subjects including controls, diabetics and obese individuals were screened for AQP7 mutations and three types of polymorphism were found; these predicted a substitution of amino acids V59L, R12C and G264V. When cRNA carrying each mutation was injected in oocytes the AQP7 protein was normally expressed. V59L and R12C were functionally normal, but G264V mutation lacked both water and glycerol permeability (26).

A homozygote carrying G264V mutation was found, having body weight and glucose tolerance normal. When subjected to physical exercise after prolonged (20h) fasting, the elevation of plasma glycerol level was blunted in this individual in spite of normal elevation of plasma noradrenaline level. This demonstrates that G264V is crucial for the function of AQP7 (21,25).

DEFICIENCY OF WATER CHANNEL PROTEINS IN OBESITY

It seems that obesity is associated with a dysregulation of AQP7. It was discovered that KO mice developed mild obesity after 12 weeks of age; the mass of adipose tissue was large and the adipocytes were hypertrophied in KO mice compared with WT mice. Glycerol content and glycerol kinase activity of adipocytes were higher in KO mice, compared to WT mice (25,27). How are these findings related to obesity? Under normal conditions the adipose tissue exhibits only low glycerokinase activity and therefore it is believed that glycerol is not reused for lipogenesis in adipocytes (21). However, it has recently been reported that glycerol itself activates glycerol kinase by inducing conformational change in the protein (28, 29). Consequently, it obese animals produces on increased glycerol kinase activity, that, in turn leads to increased synthesis of TG and finally results in TG accumulation (21).

To confirm the effect of AQP7 deficiency or adipocytes, AQP7 was knocked down in 3T3-L1 adipocytes using RNAi. AQP7 knockdown resulted in a significant increase of intracellular glycerol levels, a 4-fold increase in glycerol kinase activity, an enhanced uptake of oleic acids and finally an increase of TG in adipocytes (26). In effect, disruption of water channel in adipocytes caused obesity (30). In lean mice, the expression of AQP7 is suppressed in the fed state. In contrast, the suppression was blunted in the visceral adipose tissue of obese mice. On the other hand, portal glycerol and systemic glucose levels were elevated in obese mice. The liver has glycerokinase activity and can activate glycerol to use it for gluconeogenesis, a pathway that is activated during fasting (21). The liver also has AQP9, an aquaglyceroporin which facilitates the uptake of glycerol. The expression of AQP9 is suppressed in the fed state of normal mice, while it was blunted in obese mice (30). In another study remarkable age-dependent adipocyte hypertrophy in AQP7-deficient mice was observed (31). WT and KO mice had similar growth at 0-16 weeks as assessed by body weight; however, by 16 weeks KO mice had 3.7 fold increased body fat mass. Adipocytes from KO mice at 16 weeks were greatly enlarged compared with WT mice. Adipocytes from KO mice also accumulated excess glycerol and TG. Plasma membrane glycerol permeability was 3 fold reduced in KO mice. These data suggest that adipocyte hypertrophy in AQP7 deficiency results from defective glycerol exit and consequent accumulation of glycerol and TG. Furthermore, the coordinated regulation of adipose-specific AQP7 and liver-specific AQP9 may be key to determine glucose metabolism in insulin resistance, suggesting that AQP7/APQ9 might be a novel adipopharmacologic targets for drug development against obesity and related diseases (30,32-34).

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