The roles of Na⁺/H⁺ exchanger regulatory factor 1 and Aquaporin-1 in the pathomechanism of experimental acute pancreatitis

Ph.D. Thesis

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Articles closely related to the subject of the thesis

I. Pallagi P*, **Balla Z***, Singh AK*, Dósa S, Iványi B, Kukor Z, Tóth A, Riederer B, Liu Y, Engelhardt R, Jármay K, Szabó A, Janovszky A, Perides G, Venglovecz V, Maléth J, Wittmann T, Takács T, Gray MA, Gácser A, Hegyi P, Seidler U, Rakonczay Z Jr. The role of pancreatic ductal secretion in protection against acute pancreatitis in mice. Crit Care Med. 2014 **[IF$_{2014}$: 6.312]**; 42(3):e177-88. PMID: 24368347. *The first three authors contributed equally.

II. Venglovecz V, Pallagi P, Kemény LV, Balázs A, **Balla Z**, Becskeházi E, Gál E, Tóth E, Zvara Á, Puskás LG, Borka K, Sendler M, Lerch MM, Mayerle J, Kühn JP, Rakonczay Z Jr, Hegyi P. The importance of aquaporin 1 in pancreatitis and its relation to the CFTR Cl(-) channel. Front Physiol. 2018 **[IF$_{2018}$: 3.201]**; 9:854. PMID: 30050452.

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1 INTRODUCTION

1.1 Structure and basic functions of the pancreas

The pancreas is made up of exocrine and endocrine parts. The exocrine pancreas secretes approximately 1.5-2 liters of isotonic fluid per day, which is rich in HCO_3^- and contains digestive enzymes. The exocrine pancreas consists of two main cell types: acinar cells (responsible for the production of digestive enzymes) and ductal cells (responsible for bicarbonate and fluid secretion), the former cells constitute the majority of glandular tissue. The ductal cells form a complex tubular network that runs through the entire gland and unites in an outlet duct (Wirsungianus).

1.2 Ductal bicarbonate secretion

The primary role of pancreatic ductal cells is the secretion of HCO_3^-. The first step in the secretion of HCO_3^- is its uptake from the extracellular space. This process occurs through the basolateral membrane via the Na^+/HCO_3^- cotransporter, or by diffusion of CO_2, which is then converted to H_2CO_3 by carbonic anhydrase. The resulting protons are released from the cell by Na^+/H^+ exchangers or H^+ pumps. Intracellular HCO_3^- derived from H_2CO_3 is secreted through transporters. This is followed by the second step of HCO_3^- secretion: HCO_3^- exits ductal cells via cystic fibrosis transmembrane conductance regulator (CFTR) and solute carrier anion exchangers (SLC26A3, or down-regulated in adenoma, DRA; or SLC26A6, putative anion transporter-1, PAT-1) and is secreted into the luminal space.

In contrast with the well-understood role of digestive enzymes, the functions and processes of pancreatic electrolyte secretion have not yet been clearly described, although it surely facilitates the transport of zymogens to the small intestine and contributes to the neutralization of stomach acid in order to provide optimal pH for digestive enzymes in the duodenum. This is significant because maintaining a neutral or alkaline luminal pH is crucial for the membrane dynamics of acinar cells and, consequently, for the exocytosis of zymogen granules. Previous studies have shown that lower extracellular pH enhances secretagogue-induced zymogen activation in acinar cells. Recent studies have also shown, as a result of the Pancreatic Research Group in Szeged, that proper HCO_3^- secretion is

essential to prevent autoactivation of trypsin in the ductal tree. Moreover, trypsin itself can reduce apical proteinase-activated receptor-2 by activating CFTR-dependent HCO_3^- secretion in ductal cells, leading to further trypsin autoactivation. Thus, the reduction of ductal HCO_3^- secretion is of paramount importance in the preservation of intact pancreatic structure.

1.2.1 The roles of CFTR and NHERF-1 in ductal secretion

CFTR is expressed in the apical membrane of ductal cells, but it is absent in acinar cells. Its proper function is essential for pancreatic ductal HCO_3^- and fluid secretion. The importance of CFTR can be illustrated by cystic fibrosis, in which the lack of CFTR-dependent fluid secretion has been shown to cause nearly complete glandular destruction at birth in 85% of patients.

The intercalated and/or intralobular and interlobular ductal cells express CFTR (to varying degrees depending on the species) and are responsible for producing an isotonic, highly alkaline fluid with a 140 mM $NaHCO_3$ content. However, the composition of the secretion may vary from species to species depending on the flow rate. At maximum velocity, HCO_3^- concentration is about 140 mM in most species with the exception of rats and mice, where the maximum is about 70 mM.

Na^+/H^+ exchanger regulatory factor-1 (NHERF-1), also known as Ezrin binding protein-50 (EBP50) is a cytosolic regulatory protein. The role of NHERF-1 in the pancreas is not yet well understood. However, deletion of NHERF-1 resulted in gross mislocalization of CFTR, causing marked reduction in pancreatic ductal fluid and bicarbonate secretion.

1.2.2 Role of SLC26A3/A6 in ductal bicarbonate secretion

SLC26A6 (putative anion transporter-1, PAT-1) was originally identified as a chloride transporter, but subsequent studies have shown that it functions as a $2HCO_3^-/1Cl^-$ exchanger. Although some laboratories have examined the characteristics of HCO_3^- transport in intact pancreatic ducts isolated from SLC26a6 KO mice, further investigation is required to prove the role of SLC26 anion exchangers in ductal fluid and HCO_3^- secretion.

1.2.3 Role of aquaporins in ductal fluid secretion

Aquaporins (AQPs) are small membrane proteins whose primary function is to help transport water molecules. Recent studies have highlighted their essential role in specific regulatory. To date, 13 AQP isoforms have been identified in mammals, and some show species-specific expression patterns in the pancreas. AQP1, -5, -8, and -12 are also present in the human pancreas. AQP1 is the first AQP described that is only permeable to water. The presence of AQP1 has been previously demonstrated in the apical and lateral plasma membranes of centrifugal cells and the apical and basolateral membranes of intercalary and intralobular ductal cells. AQP5 has been proven to be an aquaglyceroporin that aids in the transport of water along with glycerol, urea, and other small solutes. The expression of AQP5 was not detected in the centroacinar cells but only in the apical plasma membrane of the intercalated ducts. The colocalization of AQP1 and -5 with CFTR on the apical membrane of the ducts is of particular interest, confirming that the channels influence each other's function.

1.3 Acute pancreatitis

Acute pancreatitis (AP) is a sudden inflammation of the pancreas, the severity of which ranges from a mild to a severe, the latter of which sometimes has a fatal outcome.

1.3.1 Clinical features

1.3.1.1 Incidence and etiology

The incidence of AP varies from country to country. In Western-type societies, it is most commonly 15-45/100 000 inhabitants/year, which is still slightly increasing. AP is one of the most common gastroenterological disorders requiring hospital admission. In fact, this means 200 000 hospital admissions in the US each year, costing the health care system $ 2-2.5 billion.

Alcohol consumption and gallstones are responsible for the development and exacerbation of 70-80% of AP cases. However, several other etiological factors (e.g. drugs, hypertriglyceridemia, pancreatic- or ampulla of Vater tumor, trauma, various mumps-, H1N1-, Coxsackie virus, bacterial infections) are known. In 10-20% of cases, the cause cannot be identified.

1.3.1.2 Classification

In 2011, the revised Atlanta-classification was created for the most accurate diagnosis, evaluation of severity, and classification of AP. On the basis of the development and duration of organ failure, mild, moderate and severe AP can be distinguished. Approximately 75-80% of cases are mild, 15-20% moderate, and about 10% are severe. In mild AP, no local or distant complications occur. Patients with mild AP have a mortality rate of less than 1%, it is around 5% with moderate pancreatitis, and 30% to 50% with severe AP.

1.3.1.3 Clinical symptoms and treatment

One of the most common symptoms of AP is abdominal pain, usually in the epigastrium, which radiates to the back. The treatments given to the patient are mainly symptomatic, e.g. fluid replacement, nutrition, analgesia, reduction of pancreatic secretory function when needed, and antibiotics if an appropriate indication is present. Enteral nutrition can be either nasogastric (currently under investigation) or nasojejunal (classically accepted mode of nourishment).

1.3.2 Pathomechanism

The pathomechanism of the disease is not fully understood, and unfortunately, specific therapy is still unknown. Therefore, the search for new therapeutic targets, experimental, and clinical examination of inflammation is also vital for reducing the mortality of the disease. Thus, several theories are known about the pathomechanism of inflammation, the mechanisms of which have been learned from *in vivo* and *in vitro* experiments. All of which are likely to play a role in the development of human AP.

In the pancreas, various aggressive and/or protective processes play a role in mediating the course of AP. The disease, initiated by various etiological factors, is likely to run on multiple parallel strands, which can eventually lead to inflammation of the pancreas and, in severe cases, cell death. These processes include: premature activation of trypsinogen, activation of nuclear factor kappa-B (NF-κB), early acinar mitochondrial damage, the role of white blood cells, oxidative stress, and inflammatory mediators. Heat shock proteins (e.g. HSP 72) and pancreatic ductal secretion are likely to be protective.

2 AIMS

In this study, I had the following goals:

- to investigate the role of NHERF-1 protein in acute experimental pancreatitis
- to investigate the role of AQP1 channel in acute experimental pancreatitis.

3 MATERIALS AND METHODS

3.1 Ethical approval and animals

Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (2010/63/EU Directive). In addition, the experimental protocol was approved by the local Ethical Board of the University of Szeged, Hungary and also by the National Scientific Ethical Committee on Animal Experimentation (Budapest, Hungary).

Mice were housed in a typical animal care facility in regular plastic cages on 12:12 hour light-dark cycle at room temperature (23 ± 1 °C) and were allowed free access to standard laboratory chow for rodents and drinking water. All mice were genotyped prior to the experiments. Experiments were performed on litter-matched (12-16-week-old) wild-type (WT), and NHERF-1 or AQP1 knock out (KO) male mice.

3.2 Induction of acute pancreatitis

3.2.1 Cerulein-induced pancreatitis

AP was induced in mice by hourly (7 or 10 times) i.p. injections of cerulein (50 µg/kg per injection). Control mice were given PS (0.9 % NaCl) solution i.p. instead of cerulein. Two hours after the final injection, mice were euthanized by pentobarbital overdose (85 mg/kg i.p.). Mice were exsanguinated via the inferior vena cava (NHERF-1 experiments) or via cardiac puncture (AQP1 experiments), and the pancreata were immediately removed. The pancreas was trimmed from fat and lymphatic tissue.

Approximately one-quarter of the pancreatic tissue was put into a 6 % neutral formaldehyde solution, and the other three quarters were dropped in liquid nitrogen and stored at -80 °C until use. The collected blood was centrifuged at 4 °C with 2500 RCF for 15 min and the serum was stored at -20 °C until use.

3.2.2 Sodium-taurocholate-induced pancreatitis

Na-taurocholate was administered intraductally (i.d.) as described previously by Perides *et al.* Briefly, anesthesia was carried out using i.p. administration of ketamine (125 mg/kg) and xylazine (12.5 mg/kg) cocktail. The duodenum was punctured with a 0.4 mm diameter needle connected to a polyethylene tube after performing median laparotomy. Leakage of Na-taurocholate was prevented by temporary ligature of the biliopancreatic duct, while the proximal bile duct was temporarily occluded with a microvessel clip. 4 % Na-taurocholate or PS solution was infused with an infusion pump (10 μl/min) for 5 min. After the infusion, we removed the microvessel clip, the distal ligature, and also the injection needle. For a short time, the possibility of bleeding was monitored, and the abdominal wall and the skin were sewed. The body temperature of animals was monitored and was kept at 37 °C until they woke up. Mice were exsanguinated through the inferior vena cava 24 h after the i.d. injection. Thereafter, all procedures were carried out as described previously in the case of cerulein-induced pancreatitis. The only difference was that all of the pancreatic tissues were fixed in a 6 % neutral formaldehyde solution for histological quantification.

3.3 Assays and histologic examination

Amylase activity was measured with commercial colorimetric kit using a microplate reader at 405 nm. Acinar cell viability was determined by using the 0.4 % trypan-blue exclusion test. Pancreatic IL-1β concentrations were measured by ELISA. Pancreatic expression of HSP-72, IκB-α, and IκB-β contents were determined by Western-blot analysis. Myeloperoxidase (MPO) activity, as a marker of tissue leukocyte infiltration, was assessed by the method of Kuebler *et al*.

Pancreatic injury was evaluated by semiquantitative grading of interstitial edema, leukocyte infiltration, necrosis, and in case of the Na-taurocholate model hemorrhage. The extent (%) of cell damage was confirmed by analysis with ImageJ software (NIH, Bethesda,

MD, USA). The fixed pancreatic tissue was embedded in paraffin blocks, cut into 3 µm thick sections, and stained for hematoxylin-eosin using standard techniques and viewed by light microscopy. A semiquantitative scoring system (0-3) was used in the case of AQP1 experiments, as we described previously. Edema (0: none, 1: patchy interlobular; 2: diffuse interlobular; 3: diffuse interlobular, and interacinar), leukocytic infiltration (0: none; 1: patchy interlobular; 2: diffuse interlobular; 3: diffuse interlobular and interacinar). The rate of necrosis was expressed as a percentage of the total analyzed pancreatic area.

3.4 Statistical analysis

Statistical analysis was performed by SigmaPlot (Systat Software Inc., Chicago, IL, USA). Data are presented as means±SEM. Both parametric (one- or two-way analysis of variance (ANOVA)) and non-parametric (Kruskal-Wallis) tests were used based on the normality of data distribution (analyzed by the Shapiro-Wilk test). Post-hoc analysis (either Dunn's or Bonferroni's test) was performed according to the recommendations made by SigmaPlot. χ^2-test was used to determine differences between groups in the proportion of mice who died. $P < 0.05$ was accepted as statistically significant.

4 RESULTS

4.1 Cerulein-induced pancreatitis is more severe in NHERF-1-knock-out mice

To determine if the reduction of pancreatic secretion could influence the development of AP, WT or *NHERF-1* KO mice were given 10 hourly i.p. injections of either PS (control) or supramaximal doses of cerulein.

The control animals had normal pancreatic histology. I.p. injections of cerulein caused extensive pancreatic cell damage; the rates of necrosis, and apoptosis were markedly higher in the *NHERF-1*-KO vs. WT mice. However, no significant differences were observed in the extent of interstitial edema (2.0 ± 0.11 for WT vs. 2.2 ± 0.2 for KO) or leukocyte infiltration (1.72 ± 0.08 for WT vs. 1.95 ± 0.13 for KO, p=0.08) in cerulein-treated groups.

Serum amylase activities were significantly elevated in cerulein-treated vs. control WT and *NHERF-1*-KO mice. Importantly, amylase activity was significantly higher in the cerulein-treated *NHERF-1*-KO vs. WT mice. Pancreatic MPO activities were significantly increased in cerulein-treated vs. control groups, but they were not different in WT compared to KO mice exposed to cerulein. Pancreatic HSP-72 expression was significantly increased in cerulein-treated vs. control groups, and significant differences were also observed between cerulein-treated WT and *NHERF-1*-KO groups.

Key events in the pathogenesis of AP include premature activation of pancreatic trypsinogen and the activation of the proinflammatory transcription factor NF-κB. To exclude any potential effects of *NHERF-1* deletion on early trypsinogen and NF-κB activation (regulated by IκBs), we measured pancreatic trypsin activity, and expression of IκBs in mice injected i.p. with 1x50 μg/kg cerulein. Trypsin activity was increased by about 4-fold 0.5 h after the injection of cerulein compared to the control group; however, there were no significant differences between WT and *NHERF-1*-KO mice. Also, with respect to IκB-α expression, there were no significant differences between WT and *NHERF-1*-KO animals in cerulein-treated groups.

The expression of pancreatic IκB-β was significantly higher in *NHERF-1*-KO vs. WT control mice, and no differences were observed in cerulein-treated WT and NHERF-1-KO groups. These data demonstrate that the difference in AP severity between WT and *NHERF-1-KO* mice is independent of pancreatic trypsinogen and NF-κB activation.

The expression of the proinflammatory cytokine IL-1β was significantly elevated in the pancreas of cerulein-treated vs. control WT and *NHERF-1*-KO mice, but there was no significant difference between the cerulein-treated WT vs. *NHERF-1*-KO mice.

Of note, i.p. administration of 7x50 μg/kg cerulein in WT and *NHERF-1*-KO mice caused similar effects in the investigated histological and laboratory parameters, as shown for the higher cerulein dose. Overall, our results clearly demonstrate that the severity of cerulein-induced AP is lower in mice expressing NHERF-1.

To exclude any possible deleterious effects of *NHERF-1* deletion on cholecystokinin receptor function, we tested the sensitivity of acinar cells to cerulein.

Amylase secretion of acinar cells from WT and NHERF-1-KO animals showed no significant differences in response to cerulein stimulation.

4.2 Intraductal administration of sodium-taurocholate causes more extensive acinar cell necrosis in NHERF-1-KO compared to wild-type mice

We also investigated if *NHERF-1*-KO mice respond differently than WT mice when AP was induced by i.d. infusion of 4 % Na-taurocholate. Postoperative mortality after administration of Na-taurocholate in KO mice (2/14 animals) was not significantly different vs. WT animals (0/10).

Intraductal infusion of PS caused no postoperative mortality, but mild pancreatic edema and inflammation were seen on histology without significant necrosis. The rate of leukocyte infiltration was significantly higher in the PS-treated *NHERF-1*-KO vs. WT mice. The infusion of 4 % Na-taurocholate into the pancreatic duct induced necrotizing AP in the head), but not in the tail of the pancreas (not shown). The latter finding is in accord with that of others. Therefore; only the pancreatic heads were used for histological analysis. Approximately 24 % of acinar cells were necrotic in WT and about 47 % in *NHERF-1*-KO mice. To summarize the histopathological changes in the various groups: significantly higher rates of leukocyte infiltration were detected in Na-taurocholate-treated vs. PS-treated WT groups. In contrast, there were no significant differences in leukocyte infiltration between the Na-taurocholate vs. PS-treated *NHERF-1*-KO groups, and Na-taurocholate-treated *NHERF-1*-KO vs. WT groups.

Serum amylase activities were significantly higher in Na-taurocholate-treated vs. control WT and *NHERF-1*-KO groups, but there were no differences between Na-taurocholate-treated *NHERF-1*-KO and WT mice. We did not observe any significant differences in pancreatic MPO activity between WT and *NHERF-1*-KO mice after i.d. PS infusion. However, MPO activity was increased in Na-taurocholate-treated *NHERF-1*-KO vs. control mice and was even higher in the KO compared to the WT Na-taurocholate-treated mice. Pancreatic IL-1β expression was elevated in Na-taurocholate-treated WT and *NHERF-1*-KO mice vs. the control groups. However,

there were no significant differences in the levels of IL-1β of Na-taurocholate-treated WT and *NHERF-1*-KO animals.

These data indicate that NHERF-1 expression reduces Na-taurocholate-induced pancreatic injury, but it does not necessarily influence other laboratory parameters of the disease.

4.3 The severity of acute pancreatitis is aggravated in aquaporin-1 KO mice

The pancreas had normal histology in both WT and *AQP1* KO animals. In order to test the hypothesis that AQP1 may be involved in the pathomechanism of pancreatitis, we investigated the severity of cerulein-induced AP (10x50 μg/kg i.p.) in WT and *AQP1* KO mice. I.p. injections of cerulein caused extensive cell damage both in the WT and KO animals. The extent of pancreatic necrosis was markedly greater in the *AQP1* KO (25 ± 2.8 %) vs. WT mice (12.1 ± 3.2 %), whereas no significant differences were observed in the extent of edema and in the infiltration of inflammatory cells.

The rate of pancreatic necrosis was more extensive in *AQP1* KO vs. WT mice. Furthermore, serum amylase activities were significantly higher in KO (1605 ± 6 U/l) vs. WT mice (1285 ± 51 U/l) after the induction of AP. Overall, these results indicate that in the absence of AQP1 expression, the course of pancreatitis is more severe.

5 DISCUSSION

Until quite recently, the pathophysiological relevance of pancreatic ducts in AP has been neglected. It is commonly assumed that the primary target of all stressors is the acinar cells since they are damaged in all forms of AP. However, both clinical and experimental data suggest that pancreatic ductal cells may also have fundamental roles in the development of AP. Pancreatic fluid secretion is greatly increased at the initiation of AP. Also, *in vitro* administration of agents inducing AP such as ethanol, bile acids or viruses to pancreatic duct cells stimulate bicarbonate secretion. Our hypothesis was that ductal secretion serves to defend the pancreas by washing out toxic agents such as activated

digestive enzymes when NHERF-1 protein is present. If this ductal defense mechanism is insufficient, ductal secretion will be inhibited, and the harmful enzymes cannot leave the pancreas, so it leads to more severe AP. The beneficial effect of ductal fluid hypersecretion is indicated by the fact that secretin, a major mediator of pancreatic ductal secretion, has been shown to protect against cerulein-induced AP. Furthermore, there is accumulating evidence that impaired pancreatic fluid secretion plays a role in the pathomechanism of pancreatitis.

In the present study, we investigated the roles of NHERF-1 and AQP1 (both of which have essential roles in pancreatic ductal secretion) in experimental AP.

5.1 The role of NHERF-1 in acute experimental pancreatitis

In the related article, we have demonstrated that NHERF-1 mRNA is highly expressed in mouse pancreatic ducts. Furthermore, the genetic deletion of *NHERF-1* greatly reduced the expression of CFTR in the luminal ductal cell membrane and also decreased both *in vitro* and *in vivo* pancreatic bicarbonate and fluid secretion. This may contribute to significantly increased severity of acute necrotizing pancreatitis in *NHERF-1*-KO mice in two distinct models. Importantly, early acinar events associated with AP, like trypsinogen and NF-κB activation, were unaltered by genetic deletion of *NHERF-1*. However, late events such as apoptosis and necrosis were increased in the KO animals. Notably, the genetic deletion of *NHERF-1* had no deleterious effects on functions of acinar and inflammatory cells, indicating that increased severity of the disease is precisely due to impaired ductal secretion.

Our study does not differentiate between a loss of HCO_3^- and fluid secretion via CFTR or via a disruption between CFTR and other transporters involved in pancreatic bicarbonate and fluid secretion, such as solute carrier family 26 (SLC26) anion exchangers. The reduced expression of CFTR in the apical membrane in NHERF-1-KO pancreatic ducts thus will likely decrease the activities of PAT-1 and DRA.

Several groups have shown that the binding of CFTR to NHERF may regulate CFTR activity. The unusually high expression of NHERF-1 and CFTR in pancreatic ducts is quite different from that found in the small intestine. These findings suggest to us that CFTR-NHERF-1 interaction may be crucial to pancreatic ductal secretion.

A striking finding of our study was that the severity of cerulein-induced acute necrotizing pancreatitis was significantly higher in animals lacking NHERF-1 (and thus reduced pancreatic ductal secretion), which suggests that normal ductal secretion in WT mice protects acinar cells against necrosis and apoptosis. This effect was independent of a change in cerulein sensitivity of acinar cells and shows that NHERF-1 expression is not necessary for cholecystokinin receptor function. As *CFTR*-KO mice exhibited constitutive overexpression of pancreatic proinflammatory mediators, this is not that surprising. To confirm that the effect of diminished secretion on AP severity was not specific to the cerulein-induced model, we also determined disease severity in the clinically more relevant Na-taurocholate model. Similar to the results observed in the cerulein-induced pancreatitis model, the degree of acinar cell damage in Na-taurocholate-induced AP was significantly greater in *NHERF-1*-KO versus WT mice. Our data indicate that KO animals may even be more sensitive to increased ductal pressure.

Importantly, NHERF-1 expression did not influence the degree of the cell damage caused by high concentrations of cerulein or sodium taurocholate in isolated acini. These data indicate that the general deletion of NHERF-1 does not affect acinar damage caused by the latter agents. Therefore, it is likely that factors other than variations in the direct effects of cerulein or sodium taurocholate on acinar cells are responsible for the differences in AP severities of WT and *NHERF-1*-KO mice.

5.2 The role of aquaporin-1 channel in acute experimental pancreatitis

AQP1 is a water channel that is extensively expressed in pancreatic acinar and ductal cells that are involved in fluid secretion. Altered expression or localization of AQPs is associated with different gastrointestinal disorders, such as gastritis or diarrhea; therefore, many studies have been conducted to identify the specific role of particular AQP isoforms. Although AQP1 plays an essential role in pancreatic physiology, its function under pathological conditions is not known. In order to determine the role of the AQP1 channel in AP, we used *AQP1* KO mice. In the present study, we showed that the decreased expression of AQP1 in pancreatic ductal cells induces more severe pancreatitis in a cerulein-induced AP model.

There are contradictious data partly in contrast with previous observations demonstrating that defect in AQP1 expression cause only a small but not significant decrease in the rate of stimulated pancreatic fluid secretion. Although this discrepancy can be explained by the different methods used for the measurement of pancreatic fluid, nevertheless, other studies have found that decreased expression or function of AQP1 dramatically reduces ductal fluid secretion. A direct interaction between AQPs and CFTR has also been observed in Sertoli cells.

The deletion of *AQP1* itself does not damage the pancreas and does not cause pancreatitis in mice, which might be due to the compensating effect of AQP5; however, it induces a more severe disease progression. The involvement of AQP1 in the pathophysiology of pancreatitis has already been raised previously. The importance of AQP1 in the exocrine pancreas has also been confirmed by the fact that this water channel is abundantly expressed in the zymogen granules of acinar cells and plays an essential role in zymogen swelling and probably secretion. All of these previous observations indicate that AQP1 has essential roles in both ductal and acinar functions and makes the pancreas more sensitive for AP.

6 SUMMARY OF NEW FINDINGS

1. NHERF-1 and AQP1 have profound effects on the outcome of acute pancreatitis.
2. The severity of AP, particularly acinar necrosis and apoptosis was higher in *NHERF-1* KO mice than in WT animals.
3. We have found that the deletion of *AQP1* makes the pancreas more sensitive to pancreatitis.

formázott: Normál, Nincs felsorolás vagy számozás

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"Per aspera ad astra" (Latin phrase)