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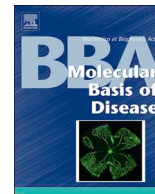
Atrial natriuretic peptide attenuates endoplasmic reticulum stress in experimental acute pancreatitis

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ABSTRACT

Increasing evidence shows that the endoplasmic reticulum (ER) stress is an early event that injures pancreatic acinar cells and contributes to the pathogenesis of acute pancreatitis. In the present work we sought to establish whether atrial natriuretic peptide (ANP) alleviated ER stress in rats with cerulein-induced pancreatitis. The major components of the unfolded protein response (UPR) and their downstream effectors were assessed by immunoblotting or fluorimetry and the ultrastructure of ER evaluated by electron transmission microscopy. Cross-talk with autophagy was evaluated by beclin-1 expression. ANP reduced binding immunoglobulin protein (Bip) expression (UPR major controller) which under non-stress conditions keeps inactive the stress sensor proteins: protein kinase-like ER kinase (PERK), inositol-requiring enzyme-1 (IRE1) and activating transcription factor 6 (ATF6). Although ANP did not change PERK expression it decreased p-eIF2α and enhanced downstream effector CHOP, suggesting that ANP stimulates ER-dependent apoptosis. In accordance, ANP also decreased Bcl2 expression and enhanced proapoptotic proteins Bax and Bak. The atrial peptide enhanced ATF6 expression and although it did not affect IRE1/sXBP1 signaling, it increased caspase-2 activity, also involved in ER-dependent apoptosis. Furthermore, ANP decreased beclin-1 expression. The ultrastructure of the RE revealed decreased swelling and conserved ribosomes in the presence of ANP. Present findings support that ANP alleviates ER stress in acute pancreatitis by modulating the three branches of the UPR and stimulates ER-dependent apoptosis. Gaining insights into the modulation of ER stress may help to develop specific therapeutic strategies for acute pancreatitis and/or medical interventions at risk of its developing like endoscopic retrograde cholangiopancreatography.

1. Introduction

Acute pancreatitis (AP) is a sudden inflammation of the pancreas initiated in acinar cells by mechanisms not yet completely understood. Most patients suffer a mild and self-limiting disease that resolves without serious complications, but approximately 20% of patients develop a severe AP with multiple organ dysfunction syndrome associated with high mortality rate. The clinical course and severity of AP can fluctuate fast and unpredictably and unfortunately there is no specific therapy for the disease [1].

Despite numerous studies over the past years, the inner aspects of AP pathogenesis remain elusive, and although different theories have been proposed, they only explain certain aspects of the pathogenesis.

Most studies support that premature trypsinogen activation and NF-κB activation are independent and parallel key events in the initiation of the disease [2]. However other factors like oxidative stress, impaired autophagy, mitochondrial injury, aberrant calcium signals, and endoplasmic reticulum (ER) stress, have also been shown to early injure pancreatic acinar cells and contribute to the pathogenesis of the disease [3–6]. A crosstalk between inflammation and ER stress is supported by the observation that the responses of the ER stress stimulate NF-kB activation, a key event in the early stages of AP [3,7].

The ER is critical for the proper folding, maturation and secretion of transmembrane and secreted proteins. When correct folding fails, proteins are targeted for degradation in the cytosol through ubiquitination by the ER-associated degradation (ERAD) and lysosome-mediated

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protein degradation via autophagy [7,8]. Factors like calcium depletion, oxidative imbalance or high cellular protein demand, perturb the ER causing accumulation of unfolded or misfolded proteins in the lumen, a situation known as ER stress. In this context the ER triggers a well conserved adaptive cellular mechanism known as the unfolded protein response (UPR) [9]. In the early phase of the UPR the adaptative response aims to restore protein folding homeostasis, but if cells fail to recover from ER stress, apoptosis is initiated [10]. Three ER transmembrane proteins orchestrate the UPR: protein kinase-like ER kinase (PERK), inositol-requiring enzyme-1 (IRE1) and activating transcription factor 6 (ATF6) which under non-stressed situations are associated with the chaperone binding immunoglobulin protein (BiP). Under ER stress BiP preferentially binds unfolded proteins so the stress sensor proteins become active and trigger the UPR [11]. However, some studies also suggest that unfolded proteins directly activate IRE1 [12].

The pancreatic acinar cell possesses abundant ER and exhibits the highest rate of protein synthesis as compared with other cell types. It is responsible for the synthesis, storage and release of a large number of digestive enzymes, necessary for the digestion and absorption of nutrients. The acinar cell ER is normally prone to stress, but the cell has coping mechanisms to compensate it. Several studies show that key regulators of the UPR are significantly altered early during the course of AP [6,13].

We previously reported that atrial natriuretic peptide (ANP) significantly attenuates AP in the rat. ANP reduces plasma amylase activity, premature trypsinogen activation, acinar vacuolization and necrotic areas in cerulein-induced AP [14]. Furthermore, it significantly attenuates the inflammatory response by reducing NF-kB activation and proinflammatory cytokine generation as well as neutrophil infiltration [15]. We also reported that the atrial peptide negatively modulates intracellular cAMP levels evoked by secretin in acinar cells. It stimulates cAMP efflux through multidrug resistance-associated protein type 4 (MRP4) as a regulatory mechanism in addition to phosphodiesterase activity to restrict the intracellular accumulation of the cyclic nucleotide within the acinar cell to prevent cell damage [16,17]. In this sense, we reported that enhanced cAMP induced by secretin administration aggravates AP but pretreatment with ANP attenuates the severity of the disease [14]. These findings support a protective role for ANP in the exocrine pancreas to prevent acinar cell damage either in the presence of enhanced cAMP levels or in AP.

Given that ER stress is an earlier event in the pathogenesis of AP and it cross-talks with inflammation and premature trypsinogen activation, in the present study we sought to establish whether ANP regulated the three branches of the UPR and their downstream signaling pathways in cerulein-induced AP.

2. Materials and methods

2.1. Animals

Sprague Dawley rats weighing 180–210 g from the Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires were used in the experiments. Animals were housed in steel cages and maintained at 22–24 °C in a controlled room with a 12-h light-dark cycle (light from 07:00 to 19:00 h). Experimental protocols were approved by the Animal Care Committee of the Facultad de Farmacia y Bioquímica de la Universidad de Buenos Aires (CICUAL-FFYB #4107/17). All procedures complied with the recommendations of the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health publication N85-23, 1985; revised 1996). Most reagents were from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

2.2. Experimental design

Animals were fed standard animal laboratory chow (Gepsa Feeds, Córdoba, Argentina), given water ad libitum, fasted overnight and randomly assigned to control or experimental groups. Animals were anaesthetized with ethyl urethane (1.25 g/kg ip), and AP was induced by four repetitive intraperitoneal injections of 40 μg/kg cerulein dissolved in saline at hourly intervals. ANP (1 μg/kg/h) (American Peptide Company, Sunnyvale, CA, USA) was infused by a cannula at the left jugular vein for 60 min starting 30 min before the first cerulein injection [14,15]. Saline-infused animals served as controls. Animals were killed by decapitation at 1 h after the last cerulein injection, and blood and pancreatic tissue samples were harvested for biochemical determinations, western blot assays and transmission electron microscopy studies. Animals with AP showed plasma amylase values threefold over control, whereas the other groups exhibited values as previously reported [14,15].

2.3. Western blot assays

Experimental procedures for Western blot were as previously detailed [15]. Samples (50 μg protein) were separated on SDS-PAGE gel and electro-transferred to PVDF membranes. Following blocking with 5% nonfat dry milk membranes were washed and exposed to primary antibodies overnight at 4 °C. After washing, membranes were further incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit (W4011) (Promega, Madison, WI, USA) or anti-mouse (sc-2005) or anti-goat (sc-2020) (Santa Cruz Biotechnology, Dallas, TX, USA) antibodies for 1 h at room temperature. Membranes were developed by a bioluminescent Western blotting detection system (Kalium Technologies, Buenos Aires, Argentina) and exposed to X-ray films or assessed by a digital system (GeneGnome XRQ; Syngene, Maryland, MD, USA). The following primary antibodies were used: anti BiP (PA1-014A) (Thermo Fisher Scientific; Rockford, IL, USA), anti ATF-6α (sc-166659), anti p-PERK (sc-32577), anti p-eIF2α (sc-101670), anti GADD 153 (CHOP) (sc-7351), anti Bax (sc-493), anti Bak (sc-517390), anti-procaspase 12 (sc-21747), anti IRE1α (sc-390960), anti sXBP1 (sc-32136), anti Becli-1 (sc-10086), anti ERK 1/2 (sc-94) (Santa Cruz Biotechnology, TX, USA), anti Bcl-2 (ab-117115) (Abcam, Cambridge, UK). ERK 1/2 was used as loading control [15].

2.4. Caspase 2 activity

Caspase 2 activity was determined by a fluorometric assay kit (Clontech, Mountain View, CA, USA) containing a fluorogenic substrate specific for caspase-2 (Acetyl-Val-Asp-Val-Ala-Asp-7-amino-4-methylcoumarin). The activity was determined by fluorometric detection (excitation, λ380 nm; emission, λ460 nm), and the negative control (blank, without sample) was subtracted from all samples. Results were expressed as fold increase over control.

2.5. Transmission electron microscopy studies

For ultrastructural analysis, pancreatic tissue was fixed by immersion in 4% wt/v paraformaldehyde plus 2.5% v/v glutaraldehyde in 0.1 mol/L phosphate-buffered saline (PBS) pH 7.4 for 24 h at 4 °C. After washing in PBS with 0.32 mol/L sucrose, sections were post-fixed with 1.5% wt/v osmium tetroxide in 0.1 mmol/L PBS pH 7.4 for 2 h at 4 °C. Following washing in PBS, sections were contrasted with 2% w/v uranyl acetate, dehydrated and embedded in Spurr medium kit (Ted Pella, Redding, CA, USA). Ultrathin sections were obtained with an ultra-microtome Porter Bloom MT 1 and collected in 300 mesh copper grids, contrasted with uranyl acetate and stained with Reynolds solution, and then photographed in a Gatam 1000 V coupled to a Zeiss EM109T Electron Microscope at different magnification (30,000×).

2.6. Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed by analysis of variance
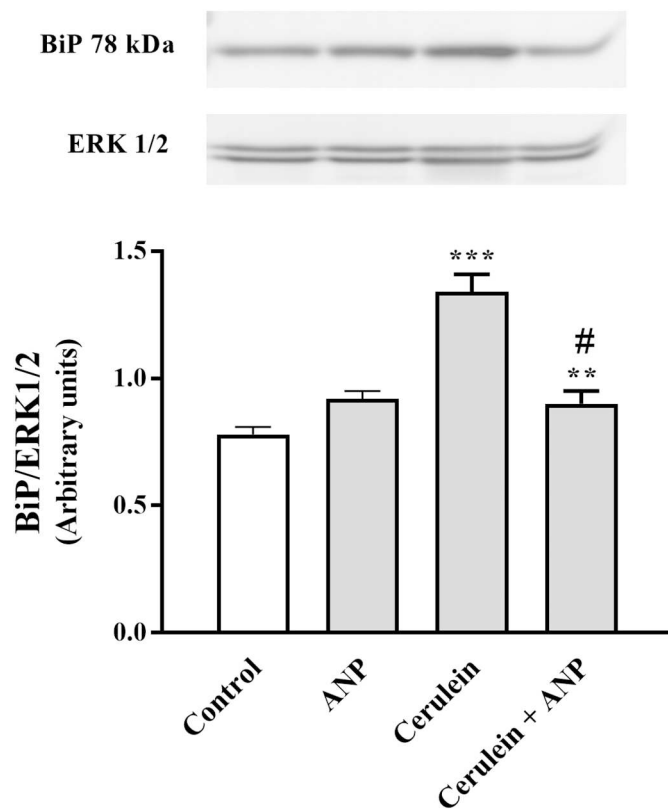
Fig. 1. ANP decreases BiP expression in acute pancreatitis. The expression of BiP was determined by western blot as detailed in Materials and Methods. Representative western blots and the densitometric analysis expressed in arbitrary units are shown. **: $p < 0.01$ and ***: $p < 0.001$ vs. control; $^{\#}$: $p < 0.05$ vs. cerulein. Number of animals per treatment: 5.

(ANOVA) followed by the Student-Newman-Keuls test. A p-value of 0.05 or less was considered statistically significant.

3. Results

3.1. ANP reduces BiP expression

BiP is the primary controller of the UPR and is highly sensitive to changes in ER status [11]. BiP expression was significantly increased in AP. However, pretreatment with ANP prevented the increase in the expression of the major RE chaperone (Fig. 1). As Bip releases the stressor proteins to assist unfolded or misfolded proteins, a decrease in this chaperone expression is indicative of less protein load to the ER.

3.2. ANP normalizes protein load to the RE and stimulates apoptosis through the PERK branch of the UPR

Activated PERK phosphorylates eIF2α leading to a general reduction in translation initiation [18]. Although activated PERK expression was not modified in any group, phosphorylation of eIF2α was increased in AP and significantly attenuated by ANP pretreatment supporting decreased protein load in the ER (Fig. 2A and B). PERK also activates CHOP which stimulates apoptosis by regulating members of the Bcl2 family [3,6]. CHOP expression was significantly increased in AP but was further enhanced by ANP supporting stimulation of ER-dependent apoptosis (Fig. 3A). In accordance Bcl2 expression, decreased in cerulein-treated rats, was further decreased when animals were also pretreated with ANP (Fig. 3B). The expression of the pro-apoptotic proteins Bak and Bax were also increased by ANP (Fig. 3C and D). These findings show that ANP stimulated ER stress-dependent apoptosis through CHOP activation.
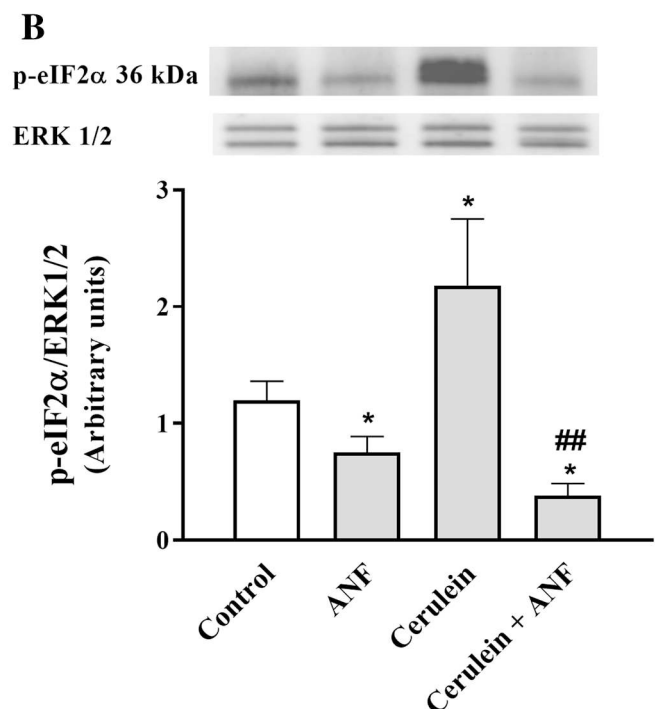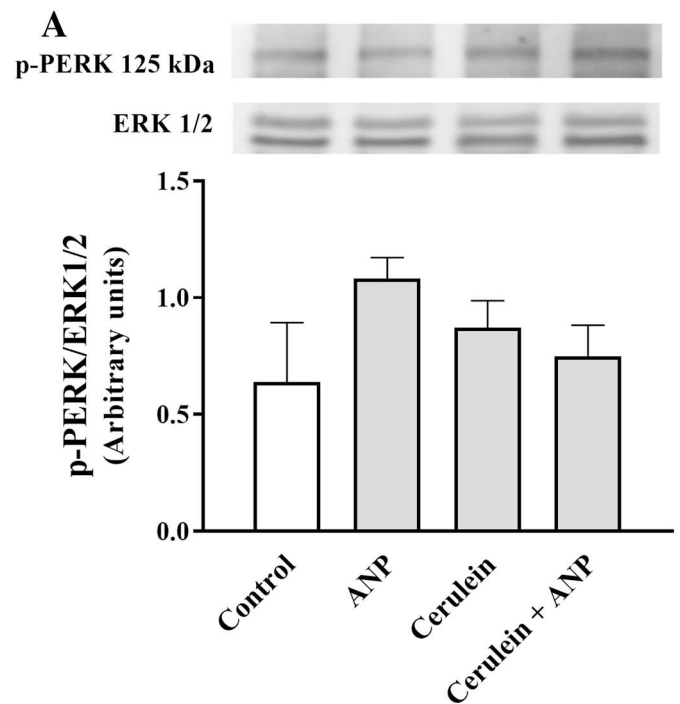

Fig. 2. ANP modulates PERK/eIF2α signaling pathway in acute pancreatitis. The expression of p-PERK (A) and p-eIF2α (B) was determined by western blot as detailed in Materials and Methods. Representative western blots and the densitometric analysis expressed in arbitrary units are shown. *: $p < 0.05$ vs. control; $^{\#\#}$: $p < 0.01$ vs. cerulein; Number of animals per treatment: 5.

3.3. ANP does not affect the IRE1-XBP1 pathway of the UPR but induces caspase-2 activation

IRE1 cleaves XBP1 mRNA to remove 26 nucleotides yielding a potent transcriptor factor sXBP1 that regulates the expression of several chaperones and foldases, causes ER expansion and attenuates protein
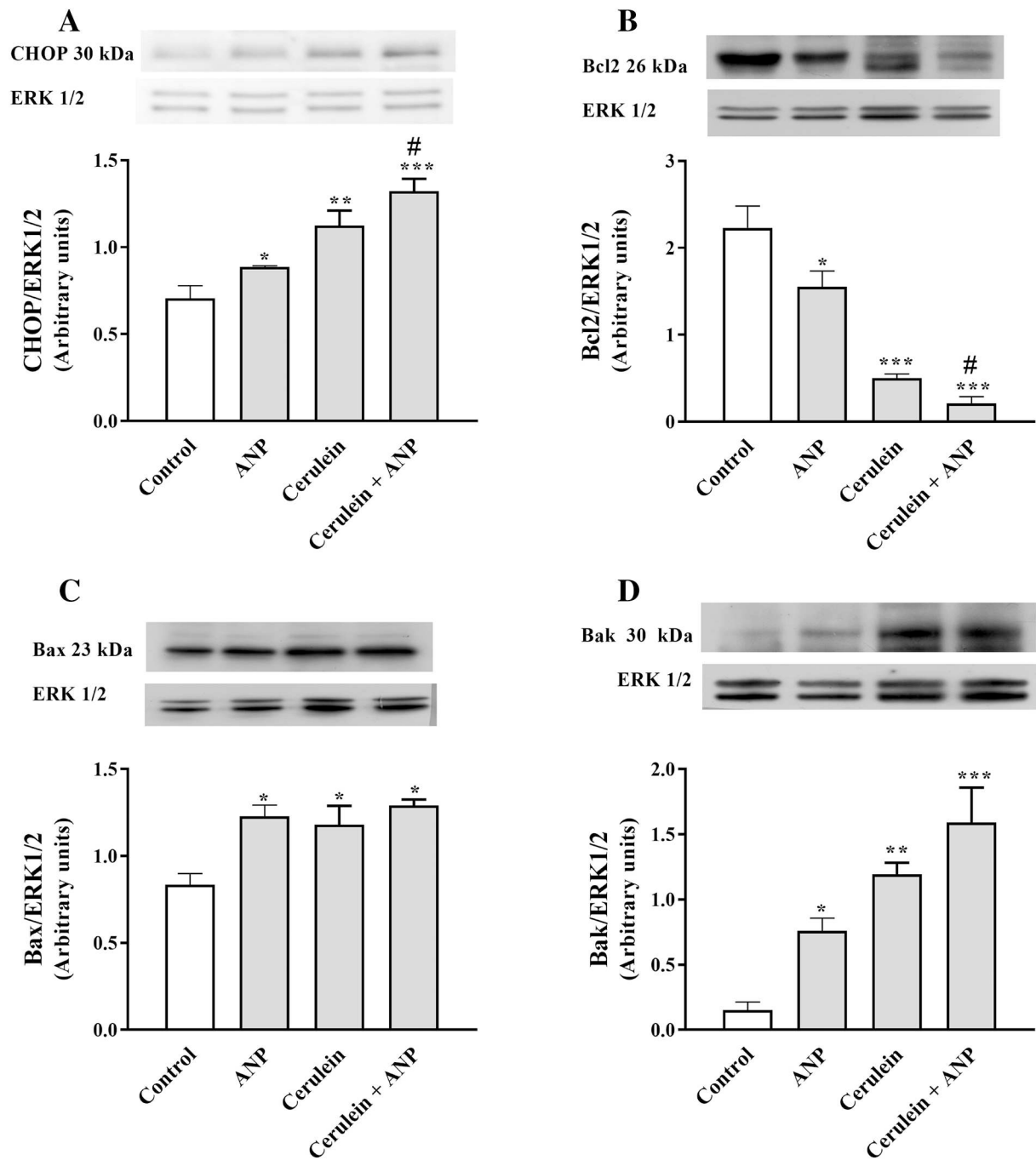
Fig. 3. ANP modulates the expression of CHOP and downstream effectors in acute pancreatitis. The expression of CHOP (A), Bcl2 (B), Bax (C) and Bak (D) was determined by western blot as detailed in Materials and Methods. Representative western blots and the densitometric analysis expressed in arbitrary units are shown. *: p < 0.05, **: p < 0.01 and ***: p < 0.001 vs. control; #: p < 0.05 vs. cerulein. Number of animals per treatment: 5.

synthesis [3,5]. Neither IRE1 nor XBP1s expression was modified by ANP in animals with or without AP (Fig. 4A and B). The stress sensor protein IRE1 also mediates a process called IRE1 dependent decay (RIDD) to reduce protein synthesis [19,20]. IRE 1 activation promotes the rapid degradation of microRNAs that target caspase-2 mRNA resulting in the enhanced expression of the enzyme [21]. Although IRE1 evaluated by XBP1s expression was unchanged by ANP, caspase 2 activity was significantly enhanced in the presence of ANP in both animals with or without AP (Fig. 4C).

3.4. ANP enhances ATF6 expression

ATF6 regulates the expansion of the ER and induces the expression of target genes related to ERAD and protein folding at the ER, like foldases and chaperones [22]. The expression of ATF6 was increased in AP, but ANP pretreatment further enhanced the expression of this sensor stress protein (Fig. 5A). Surprisingly, in normal animals ANP also increased ATF6 expression (Fig. 5A).
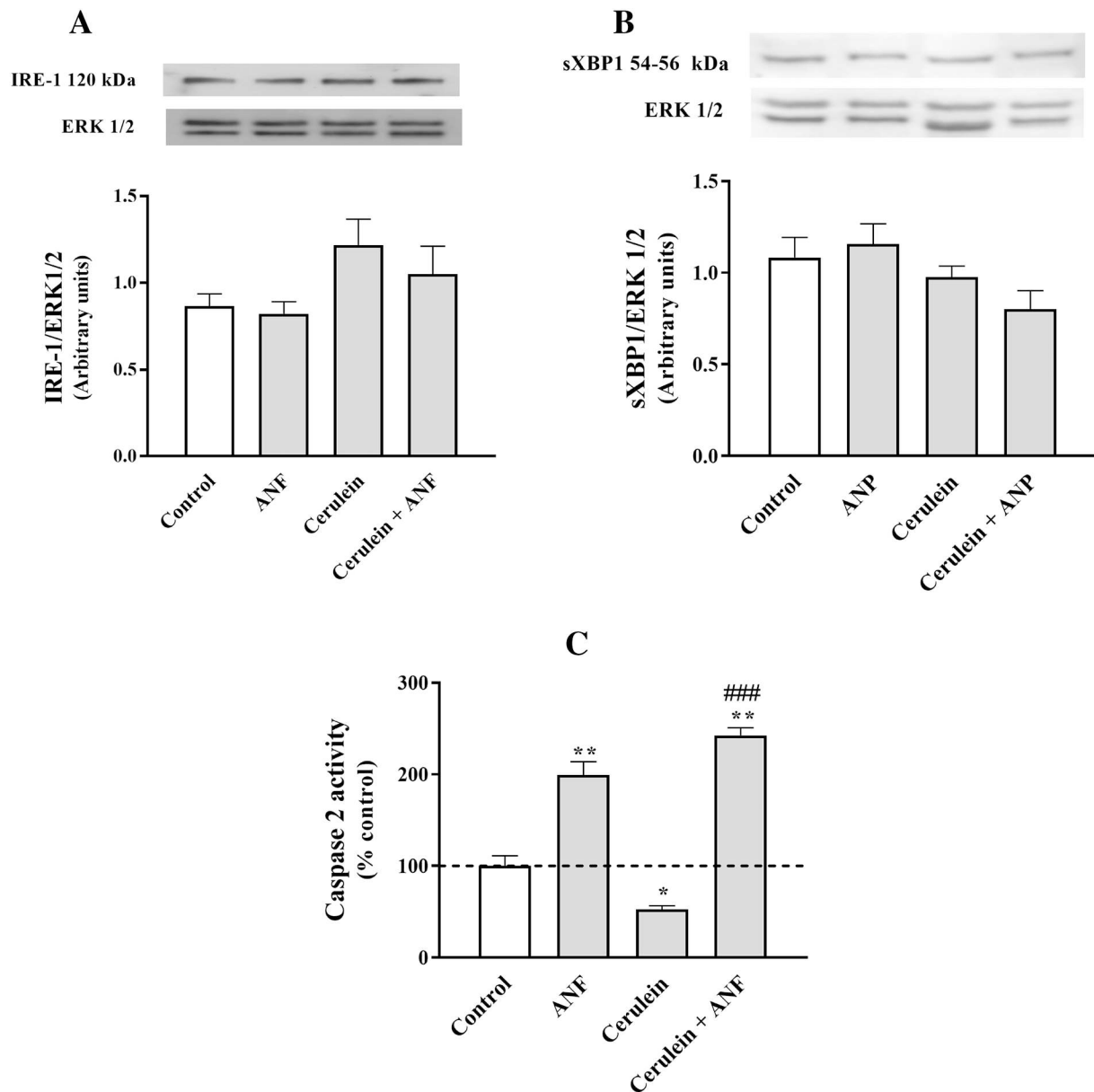
Fig. 4. ANP does not modify IRE1/sXBP1 signaling pathway but enhances caspase 2 activity. The expression of IRE1 (A) and sXBP1 (B) was assessed by western blot as detailed in Materials and Methods. Representative western blots and the densitometric analysis expressed in arbitrary units are shown. Caspase 2 activity (C) assessed by fluorometric detection (excitation, $\lambda 380$ nm; emission, $\lambda 460$ nm) as stated in Materials and Methods. *: $p < 0.05$ and **: $p < 0.01$ vs. control; ###: $p < 0.001$ vs. cerulein. Number of animals per treatment: 5.

3.5. ANP enhances caspase 12 activation

Caspase 12 is the most recently identified member of the caspase family and the only member located in the ER. It mediates ER mediated apoptosis by activating caspase 9 and 3. In the present study we measured procaspase 12 and found that its expression was decreased in cerulein-induced pancreatitis in the presence or absence of ANP, supporting caspase 12 cleavage (Fig. 5B).

3.6. ANP reduces Beclin-1 expression

Given the interplay between apoptosis and autophagy, we next evaluated Beclin-1 expression, an autophagy-related marker. Beclin-1 is a major regulator of autophagy and plays an important role in the formation of class III phosphatidylinositol 3-kinase core complex, which plays a critical role in controlling the mammalian autophagic process [23]. Increased expression of Beclin-1 was observed in acute

pancreatitis, but it was decreased by ANP (Fig. 5C).

3.7. ANP reduces ER swelling

In an attempt to increase protein-folding capacity, the UPR expands the ER size through increased biogenesis components mediated by various UPR signaling pathways. Electron microscopy studies showed swelling of the ER and loss of ribosomes in AP as previously reported. However, in the presence of ANP, the swelling of the ER was reduced, and the loss of ribosomes was not evident (Fig. 6A–D).

4. Discussion

The major finding of the present study is that ANP attenuates ER stress and stimulates ER stress-induced apoptosis in experimental AP. These findings correlate well with previous studies showing that ANP reduces the severity of AP in the rat [14,15].
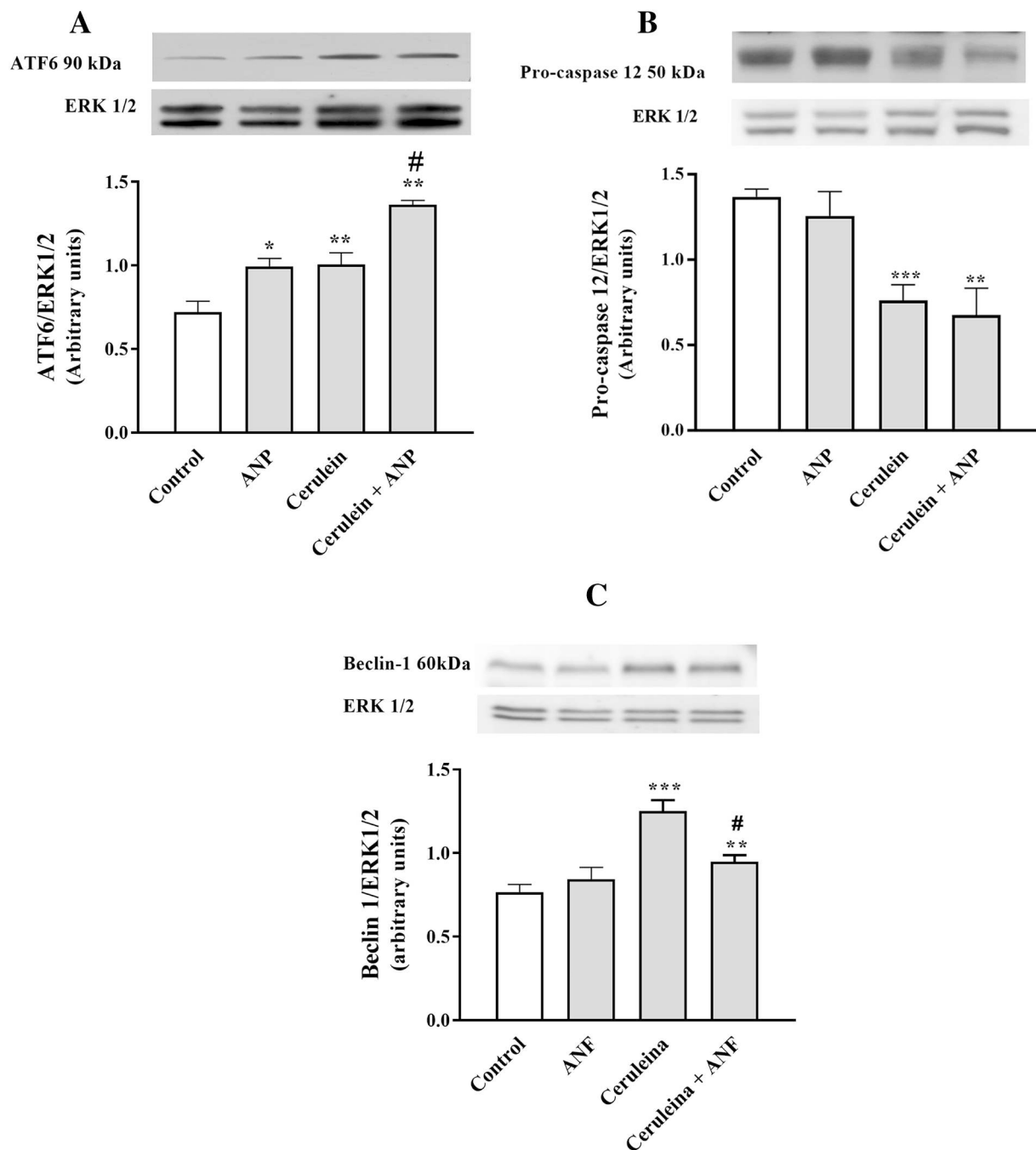
Fig. 5. ANP enhances ATF6 caspase 12 and Beclin-1 expression. The expression of caspase 12 (A) and ATF6 (B) and Beclin-1 (C) was determined by western blot as detailed in Materials and Methods. Representative western blots and the densitometric analysis expressed in arbitrary units are shown. *: p < 0.05, **: p < 0.01 and ***: p < 0.001 vs. control; #: p < 0.05 vs. cerulein. Number of animals per treatment: 5.

Accumulation of misfolded and unfolded proteins in the ER induced by calcium depletion, oxidative stress or high cellular protein demand results in ER stress which triggers the UPR. This conserved cellular response is mediated by the activation of the ER resident proteins IRE1, ATF6 and PERK with the aim to restore ER homeostasis. The UPR mechanism clears misfolded proteins from ER, reduces the flow of proteins to the ER and enhances the expression of molecules tending to protect the cell. However, if the ER homeostasis is not restored, it leads to programmed cell death [9]. Increasing evidence shows that ER stress and altered UPR signaling are emerging as key contributors of the pathogenesis of various metabolic, neurodegenerative and inflammatory diseases [24]. Strong evidence supports that ER stress responses are involved in the early stages and progression of AP [6,25]. Early

morphological changes in AP include swelling and vacuolization of the ER as well as loss of ribosomes. In this sense, key regulators of the ER stress response are significantly altered early during acute pancreatitis [13].

The components of the UPR are also involved in physiological processes like the development and differentiation of specialized secretory cells, the control of innate immunity, the control of energy metabolism and the synthesis of cholesterol and lipids. Silencing of genes encoding components of the UPR results in different outcomes [26]. The most serious is the ablation of XBP1 which triggers the collapse of the exocrine pancreas and salivary glands [27].

BiP is the major folding assisting chaperone and the primary controller of the UPR. Under normal conditions it binds to IRE1, PERK and
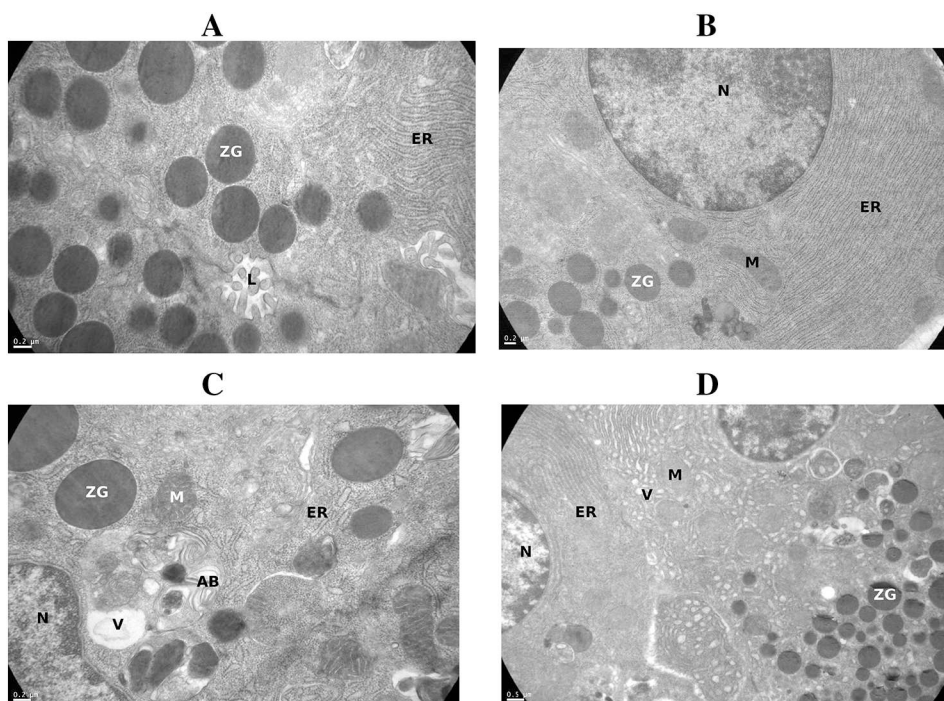
Fig. 6. ANP reduces endoplasmic reticulum swelling in acute pancreatitis. Representative transmission electron micrographs of pancreatic endoplasmic reticulum from control animals (A), treated with ANP (B), with acute pancreatitis in the absence (C) or presence of ANP (D). ER: endoplasmic reticulum; L: lumen; M: mitochondria; N: nucleus; V: vacuole; ZG: zymogen granule; AP: apoptotic body. Number of animals per treatment: 5.

ATF6 but under ER stress BiP dissociates from the stress sensor proteins to bind the unfolded proteins, so triggering the UPR. BiP level is highly sensitive to changes in ER status [4]. In the present study, BiP expression was increased in AP as previously reported, but pretreatment with ANP significantly diminished it, suggesting that ANP attenuates the UPR [4,28,29].

The UPR signal transducers IRE1, ATF6 and PERK and their downstream effectors aim to restore proteostasis. Present findings show that in the presence of ANP, IRE1 and PERK expression remained unchanged, but that of ATF6 increased at the time studied. Although PERK and IRE1 did not change, changes in the downstream effectors of these signaling pathways induced by AP and ANP supports that these UPR branches were previously activated. The UPR is a temporal dynamic process where PERK and IRE1 control immediate reactions to stress before any transcriptional reaction. Then, ATF6 and also sXBP1 and ATF4 (downstream effectors of IRE1 and PERK, respectively) regulate the expression of target genes that increase the folding capacity of the ER and improve quality control and protein degradation mechanisms. If these pro survival mechanisms fail, apoptosis is triggered. The activation kinetics and signal attenuation among the sensor stress proteins may differ depending on stimuli nature and type of cells.

The PERK branch of the UPR is strongly protective at modest levels of signaling but contributes signals leading to cell death pathways. PERK is the major protein responsible for the attenuation of mRNA translation, indirectly through the inhibition of eIF2α. Under unstressed conditions PERK is kept inactive by the ER luminal binding to BiP but under ER stress the chaperone dissociates from PERK resulting in PERK oligomerization and autophosphorylation [9]. In the present study, we used a phospho-specific PERK antibody to evaluate its activation status following cerulein administration in the presence or absence of ANP. A previous study shows that PERK is not activated in cerulein-induced AP [29]. Nevertheless, whether PERK was earlier modified by ANP, it cannot be excluded, given that downstream effectors of the PERK pathway like eIF2α and CHOP expression were changed in AP and by ANP pretreatment.

PERK phosphorylates eIF2α leading to the inhibition translation and the reduction of protein load to the ER. However, some mRNA containing short open reading frames in their 5′ untranslated regions are preferentially translated like the transcription factor ATF4 which regulates the expression of CHOP, a transcription factor that controls genes involved in apoptosis and GADD34 which leads to eIF2α dephosphorylation [10,30–33]. In the present study, ANP reduced eIF2α phosphorylation suggesting that protein translation has been resumed. Furthermore, it also enhanced CHOP expression supporting stimulation of ER-dependent programmed cell death. CHOP inhibits the expression of the gene encoding Bcl2 thus leading to the activation of the proapoptotic proteins Bax and Bak. In line with enhanced ANP-induced CHOP expression in AP we found that ANP reduced Bcl2 expression and upregulated proapoptotic Bax and Bak. These findings correlate well with previous studies showing that ANP stimulates caspase 3 activation and expression [15].

ER-dependent apoptosis is not only mediated by CHOP but also by caspase 12 and caspase 2 activation. How the UPR switches its signaling from an adaptative reaction to the stimulation of cell death is poorly understood. Caspase 12 is the only member of the caspase family, which is in the cytoplasm of the ER and can act on both ER and mitochondria [34]. Caspase 12 is activated by calpain, a cytosolic protease which translocates to the cell membrane upon calcium increase [35]. In AP caspase 12 was activated, but not further activation was observed in the presence of ANP. However, caspase 2 activity was enhanced by ANP in rats with or without pancreatitis. It was shown that caspase 2 activates the proapoptotic protein BID which in turn activates Bax and Bak [36].

Apoptosis attenuates the severity of acute pancreatitis given that it inversely correlates with necrosis [37,38]. Switching necrosis to apoptosis results in a better outcome of the disease. In previous studies we showed that ANP stimulates caspase-3 activity and expression as well as cleaved PARP-1, an early substrate of the enzyme, which is able to switch the type of cell death from apoptosis to necrosis [15]. Furthermore, it also significantly increased the number of apoptotic nuclei as revealed by TUNEL assay [15]. In this study we show that ANP stimulates ER-dependent programmed cell death.

Present results show that in AP the ATF6 branch of the UPR remains activated in the presence of ANP. ATF6 activation (involved in the reprogramming of gene expression) represents with ATF4 (upstream effector of CHOP) the second phase in the adaptative response controlled by the UPR [3]. Upon activation, ATF6 is delivered to the Golgi apparatus where it undergoes proteolytic cleavage to yield a fragment,

which then moves to the nucleus to promote the transcription of genes involved in ERAD and protein folding [39]. In AP the expression of ATF6 remained further increased in the presence of ANP suggesting that the atrial peptide stimulates ERAD and the ER folding capacity. Surprisingly, ATF6 was enhanced in normal animals infused with ANP. In this sense, it was reported that selective activation of ATF6 triggers ER expansion under conditions in which there is no indication of stress likely to protect it from potential protein overload [40]. These findings suggest that ANP-induced ATF6 activation would be beneficial for the pancreatic acinar cell which is prone to protein overload.

The IRE1 pathway was not modified by ANP or cerulein-induced AP. IRE1 has kinase and endoribonuclease activities in its cytosolic region and uses a nonconventional mRNA splicing mechanism and/or post-transcriptional modifications of diverse substrates through RIDD to transmit UPR signaling [19,20,41]. ER stress induces oligomerization and autophosphorylation of IRE1 leading to its activation. Stimulation of IRE1 endoribonuclease domain induces the splicing of XBP1 mRNA, to yield a potent transcriptional factor, sXBP1, which also plays a critical role in normal pancreatic development [42]. sXBP1 translocates to the nucleus and enhances the transcription of genes that increase the ER size and function [42]. The observation that sXBP1 expression was unchanged in AP in the presence or absence of ANP suggests that at this point IRE1 signaling is likely turned off. In accordance, it was shown that IRE1 signaling attenuates upon sustained ER stress [43]. Furthermore, a previous study showing the time course of sXBP1 expression showed that cerulein-induced AP causes a significant increase in sXBP1 at 1 and 2 h following the first cerulein injection, but no changes are observed at 4 h [8].

IRE1 is also involved in caspase 2 activation. Under conditions of sustained ER stress, IRE1 promotes the rapid degradation of microRNAs that target caspase-2 mRNA which in turn causes rapid caspase 2 activation which contributes to apoptosis [21,44]. In addition, caspase 2 activation also ameliorates oxidative stress [45]. Caspase 2 is a highly evolutionary conserved member of the caspase family, although its role is poorly understood and controversial. Several reports suggest that caspase 2 may act as an effector or initiator caspase, although other studies suggest that it would be implicated in a complex cellular signaling pathway that leads cells to initiate apoptosis as the last resort [44]. Present findings show that ANP stimulates caspase 2 activation, although in AP its activation was diminished. A previous study showed that in cerulein-induced pancreatitis neutrophils do not affect the mitochondrial pathway of apoptosis but limit apoptosis by p53-induced caspase 2 inhibition thus favoring pancreatic necrosis [46]. We previously reported that ANP reduces neutrophil infiltration and necrosis in AP, which may account for present findings regarding caspase 2 activity [15].

Present findings support that ANP alleviates ER stress. In accordance, ultrastructural studies focusing on the ER revealed that the swelling of the ER and loss of ribosomes observed in AP, were significantly attenuated by ANP.

A cross-talk between ER stress and inflammation has been proposed. In fact, the three signaling pathways of the UPR may activate NF-kB [6]. In AP activation of NF-κB triggers the inflammatory response mediated by a plethora of proinflammatory cytokines as well as enzymes like COX2 and inducible nitric oxide synthase. However, it was reported that CHOP activation induced by PERK/eIF2α plays a negative regulatory role in inflammation [7]. In a previous study we showed that ANP reduced NF-κB activation and reduced proinflammatory cytokines. The anti-inflammatory role of ANP is further confirmed in the present work by enhanced CHOP activation.

An interplay between autophagy and apoptosis where Beclin-1 plays a major role has been reported [23]. Silencing of Beclin-1 leads to apoptosis and reduction of autophagy sensitizes cells to programmed cell death [47,48]. We here show that Beclin-1 expression was increased in AP but decreased by ANP suggesting reduced autophagy. It was reported that enhanced Bax expression reduces autophagy by

inducing Beclin-1 cleavage by caspases. In particular, it was shown that caspase 3 cleaves Beclin-1 at Asp 149 thus inhibiting autophagosome synthesis [49]. In accordance, we previously reported that ANP enhances caspase 3 activity and expression and here we show that the atrial peptide enhances Bax expression.

4.1. Conclusions

Present findings show that ANP attenuates ER stress and favors ER-dependent apoptosis in cerulein-induce pancreatitis, further supporting the protective role of the atrial peptide in the course of AP. In addition, it also appears to have a protective role in acinar cells under non-stress condition. The fact that atrial natriuretic peptide has beneficial effects in the onset of acute pancreatitis may help to develop specific therapeutic strategies for the disease and/or medical interventions at risk of its developing like endoscopic retrograde cholangiopancreatography.

Contributions

APC and ANC made acquisition, analysis and interpretation of data. MSV and LGB designed the research, supervised the project and edited the manuscript. All authors have read and approved the final manuscript.

Transparency document

The Transparency document associated with this article can be found, in online version.

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