

APLNR Silencing Attenuates Apoptosis in H9c2 cells

Ellen Thompson, Asli F. Ceylan, Jun Ren, Nair Sreejayan

University of Wyoming, School of Pharmacy, Division of Pharmaceutical Sciences & Center for Cardiovascular Research and Alternative Medicine, University of Wyoming, Laramie, WY 82071

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- ✓ Both apelin and APLNR are predominantly expressed in the cardiovascular system.
- ✓ Chronic exposure to high levels of free fatty acid (FFA) leads to insulin resistance in whole animals and humans.
- ✓ Endoplasmic reticulum (ER)-stress has been suggested to be a common pathway involved in FFA-induced insulin resistance.

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- ✓ Our **hypothesis** is that APLNR downregulation will slow cardiac myocyte apoptosis, demonstrating the role of this pathway in ER stress.

Methods

- **Cell culture:** H9c2 neonatal cardiomyocytes were cultured to 70% confluence in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS). Early passages 2-6 of the cells were used for the study. Palmitic acid (PA) was used in various concentrations (0-5 mM) for different time periods (0-6 h) to evoke ER stress in the cells.
- **SiRNA transfection:** 5 μ M siRNA solution was prepared in 1X siRNA buffer. In separate tubes positive control, negative control and test siRNA and the appropriate transfection reagent were diluted with serum-free medium. The contents of each tube was mixed by pipetting carefully up and down and incubated for 5 minutes at room temperature. The tubes including transfection agent was added to the tubes including positive control, negative control and test siRNA, and mixed by pipetting carefully up and down and incubated for 20 minutes at room temperature. Antibiotic-free complete medium was added to the tubes. Culture medium was removed from the wells, and the appropriate transfection medium and the complete medium were added to each well. Cells were incubated at 37° C in 5 % CO₂ for 48–96 hours (for protein analysis). The final siRNA concentration was 25 nM. Cells were monitored for cytotoxicity.
- **Protein Estimation:** Total protein concentration was measured by BCA method.
- **Western Blotting:** Protein levels of ER stress and apoptosis pathways as well as APLNR was assessed by SDS-PAGE followed by Western blotting.
- **Data Analysis:** Data are expressed as mean \pm SEM and statistically evaluated using Student's paired t-test. Multiple comparisons were made using ANOVA followed by Dunnet's post hoc test using Sigma Plot statistical software (Jandel Scientific, San Rafael, CA). A '*p*' value of less than 0.05 was considered to be statistically significant.

Results:

1. APLNR silencing by siRNA

APLNR  **41**

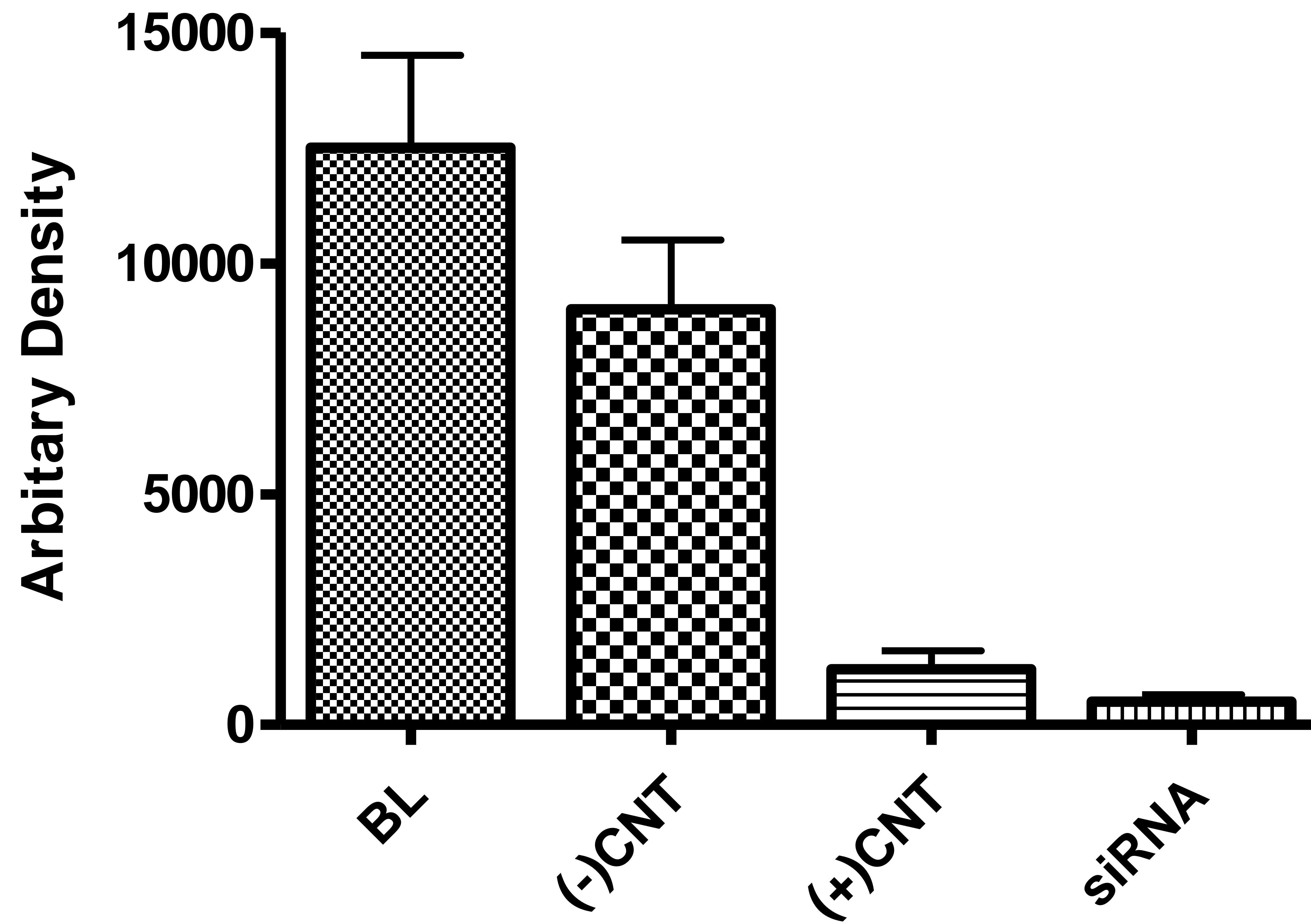
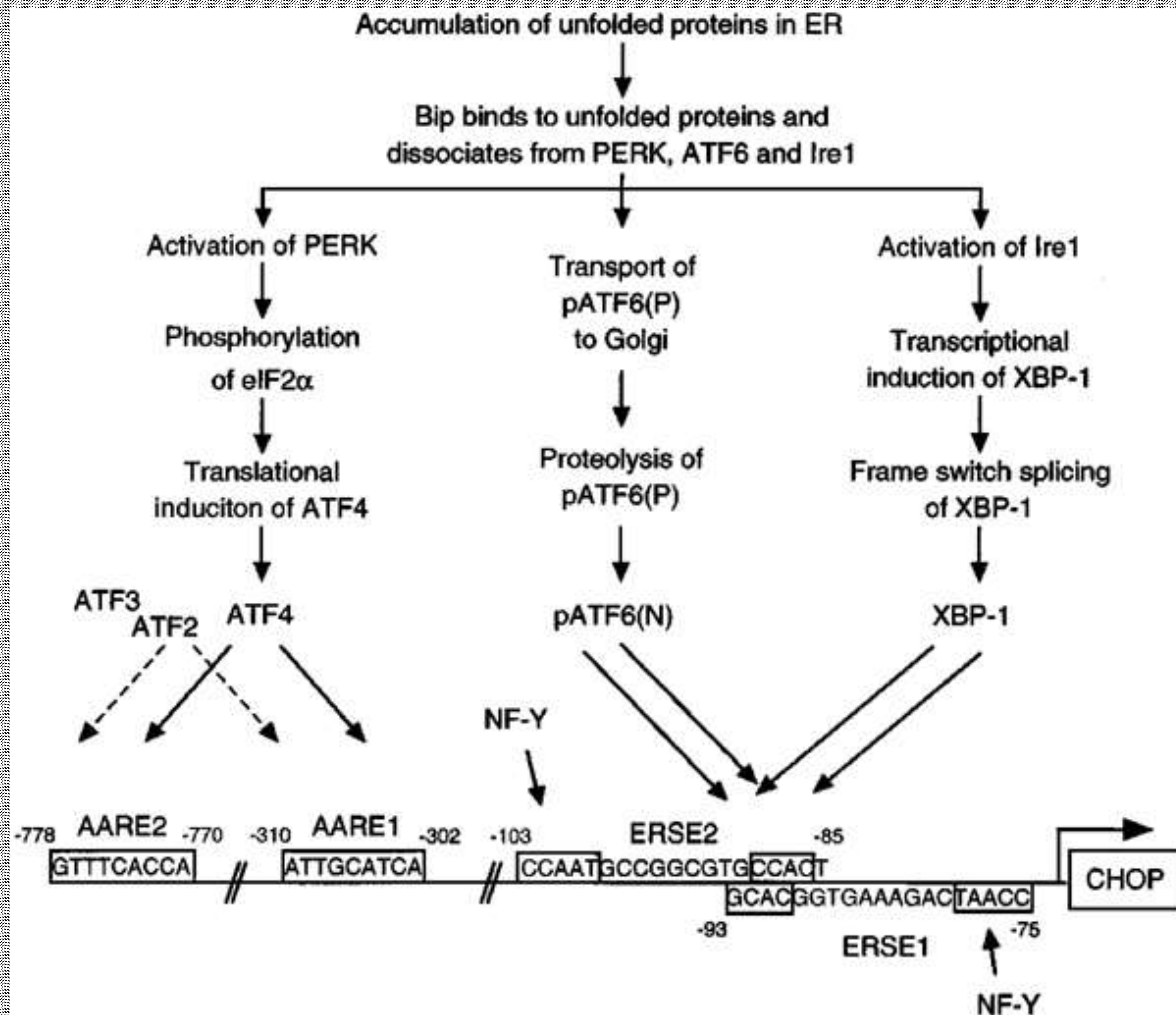


Figure 1. Western blot analysis of APLNR in H9c2 cell lysates. Cells were incubated with positive control, negative control and test siRNA for 48h. Blots show actual gel blotting using anti-ALPNR



- From the Journal, Nature

2. PA induces ER stress and APLNR in H9c2 cells.

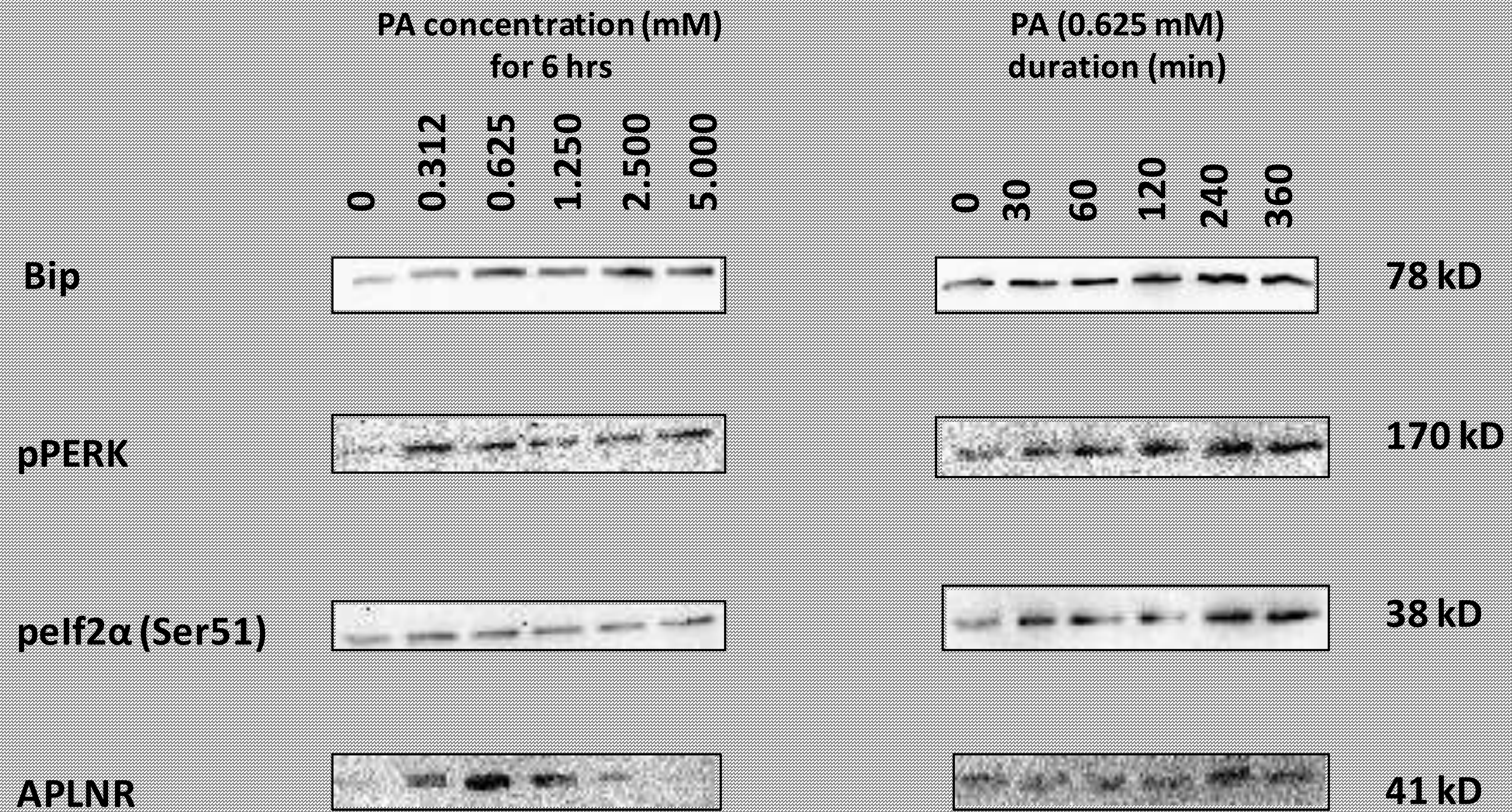


Figure 2. Western blot analysis of ER stress markers and APLNR in the presence of PA in H9c2 cell lysates. Blots show actual gel blotting using anti-Bip, anti-pPERK, anti-peIf2alpha.

3. PA induces apoptosis in H9c2 cells

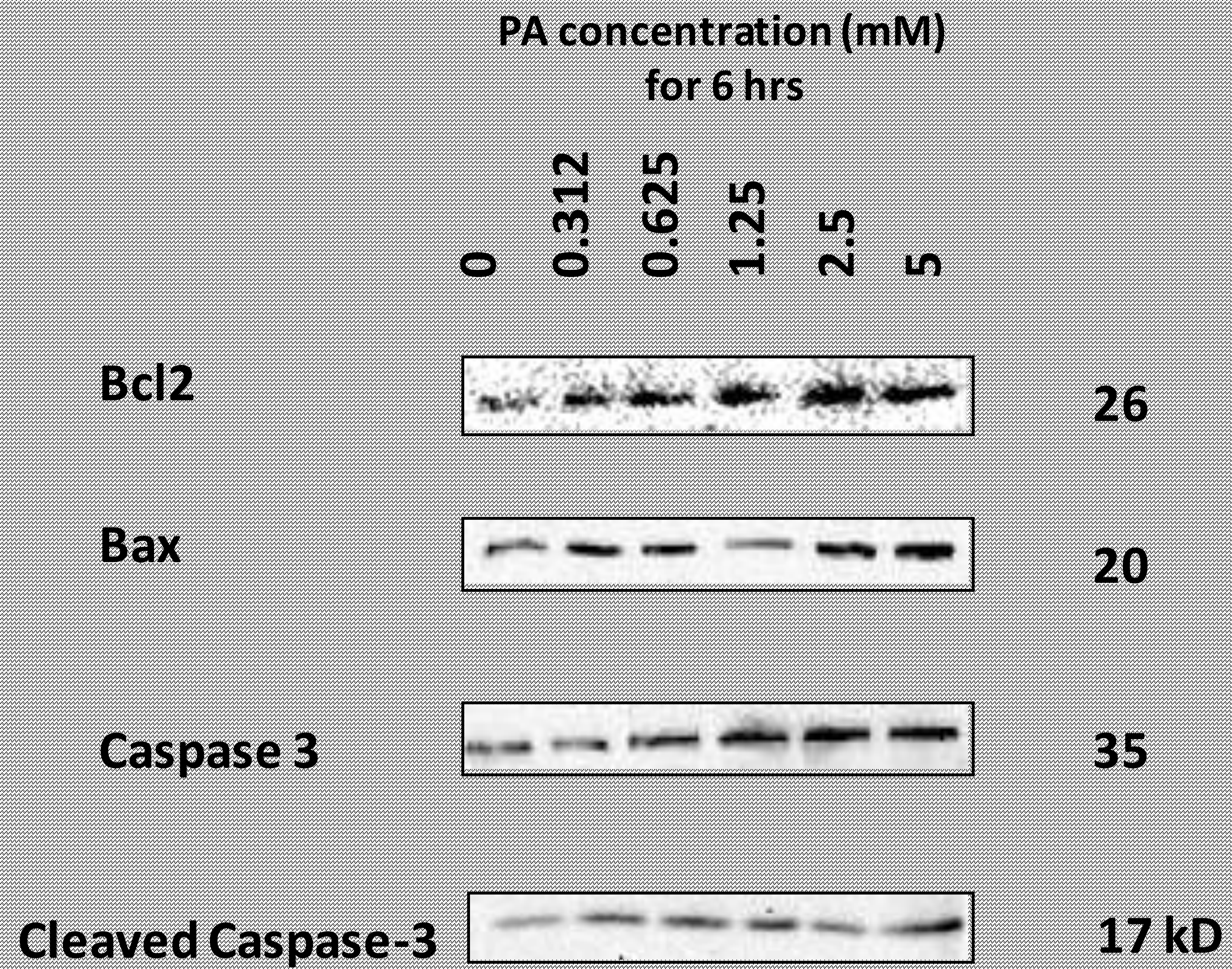


Figure 3. Western blot analysis of apoptotic markers in H9c2 cell lysates. Blots show actual gel blotting using anti-Bcl2, anti-Bax, anti-Caspase-3 and anti-Cleaved Caspase-3.

4. APLNR contributes PA-induced apoptosis in H9c2 cells.

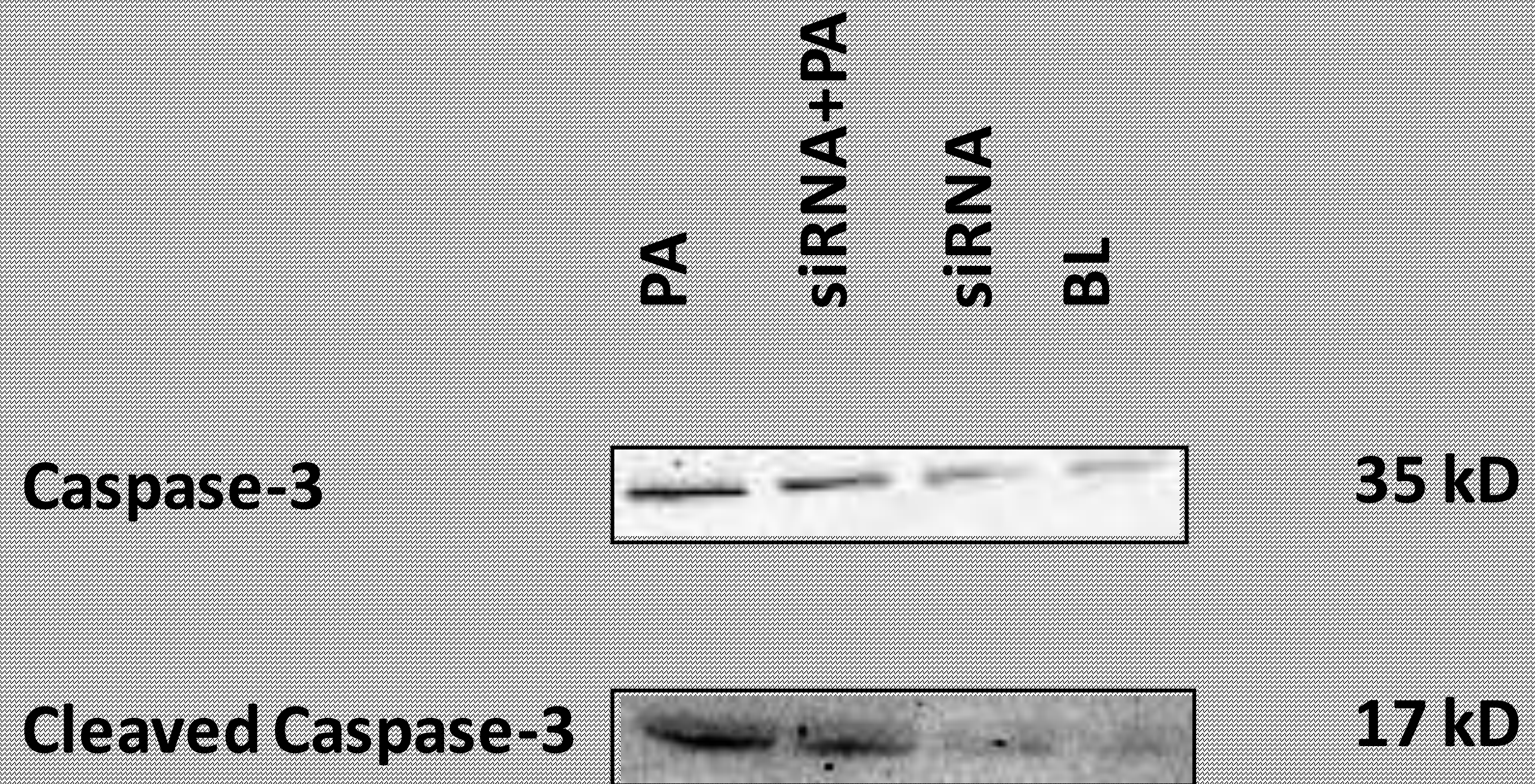


Figure 4. Western blot analysis of apoptotic markers in the presence and absence of APLNR in H9c2 cell lysates. Blots show actual gel blotting using anti-Caspase-3 and anti-Cleaved Caspase-3

Summary and Conclusion:

- Treatment of cardiomyocytes with PA induces ER stress and apoptosis
- Silencing APLNR attenuates PA-induced apoptosis in H9c2 cells.