Endocannabinoids and fertility: Anandamide induces apoptosis of human granulosa cells

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INTRODUCTION

The major endocannabinoid (eCBs) anandamide (AEA) has already been suggested as biomarker of reproductive potential of male and female gametes **[1]**. The endocannabinoid system (ECS) has been recognized as a crucial player in human reproduction and changes in AEA levels affect reproductive events **[2]**. Cannabinoid-receptor 1 (CB1) was already identified in the granulosa cells (GC) of antral follicles, and in the luteal cells of functional corpus luteum of rat ovary **[3]**. GC interact directly with the oocyte during its development, being crucial for its reproductive potential **[4]**. However, the ECS was not characterized on COV434 nor the effects of AEA on GC depicted. Therefore, the aim of this work was to explore the effects of AEA on COV434 granulosa cell line and on human granulosa cells (hGC) obtained from follicular fluid of patients enrolled in IVF treatments.

RESULTS

Our results reveal that AEA induce a reduction on GC viability in a concentration and time-dependent manner **(figure 1).** That effect on hGC was only observed at higher concentrations of AEA. This eCB did not induce ROS release nor mitochondrial membrane potential changes **(figure 2)** on both cell models, however it induced morphological changes **(figure 3),** presenting chromatin condensation at 72h. Both cell models exhibit an increase in caspase -3/7 and 9 activity **(figure 4),** suggestive of apoptosis.

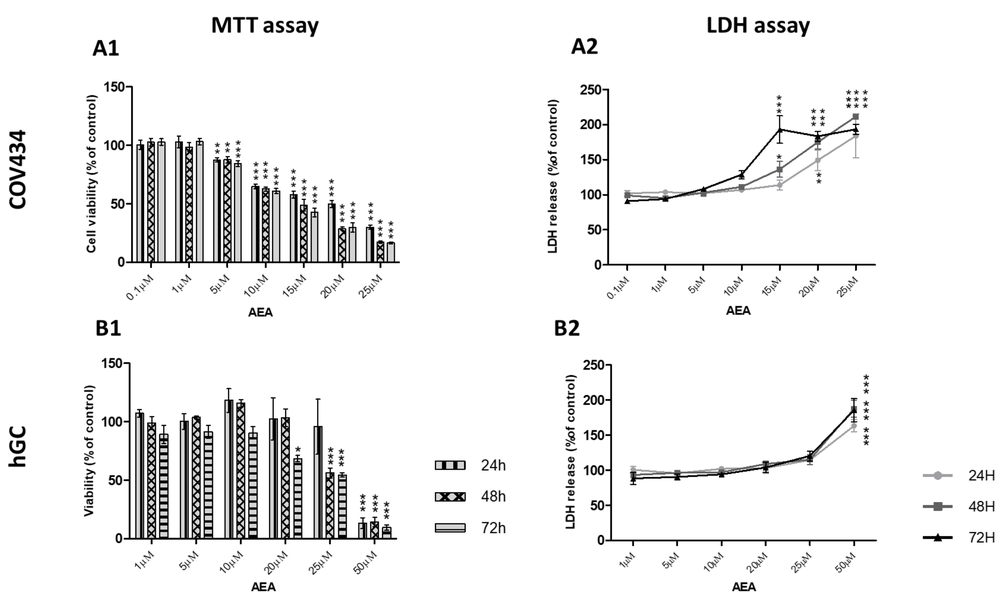


Figure 1 - Viability (A1, B1) and Citotoxicity (A2, B2) assays in COV434 (A) and hGC (B) cells after AEA exposure at 24h, 48h and 72h. Results are expressed as mean ± SEM of at least three independent experiments performed in triplicate. Significant differences between control and treated cells are denoted as *(p<0.0005), **(p<0.0005) and *** (p <0.0005).

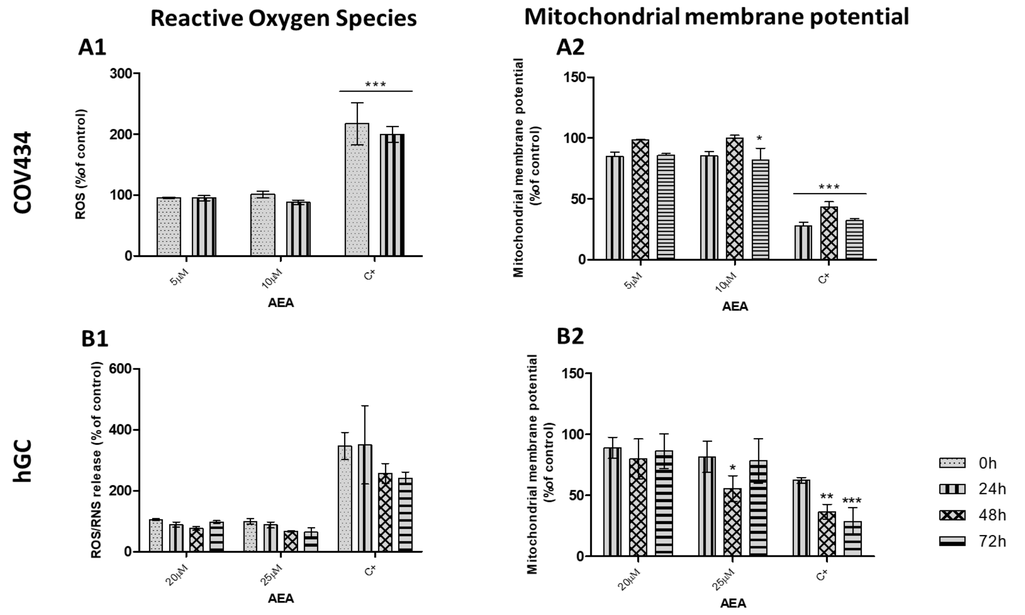


Figure 2 - Reactive Oxygen Species (A1, B1) and Mitochondrial Membrane Potential (A2, B2) after AEA exposure at different times (0h-72h) in COV434 (A) and hGC (B). H2O2 (200 µM) was used as a positive control for ROS and CCCP (10 µM) was used as a positive control for mitochondrial membrane potential. Results are expressed as mean ± SEM of at least three independent experiments performed in triplicate. Significant differences between control and treated cells are denoted as *(p<0.0005), **(p<0.0005) and *** (p <0.0005).

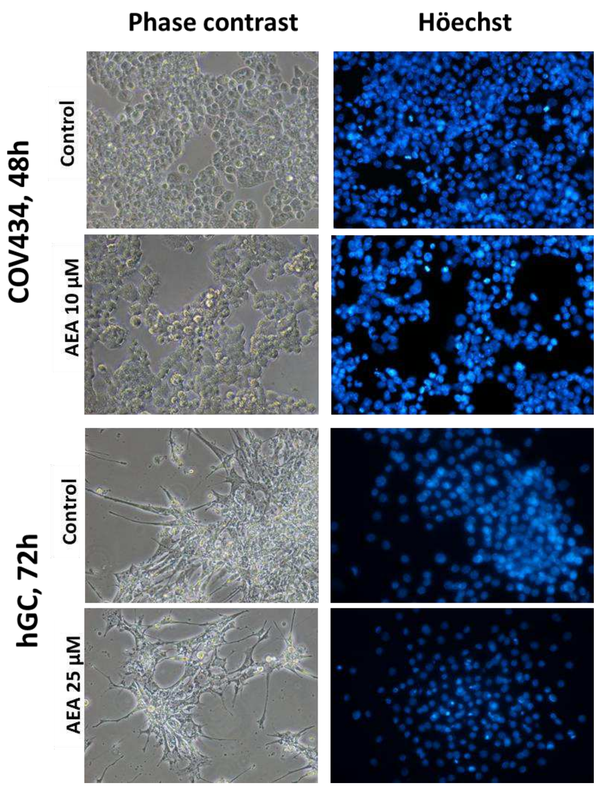
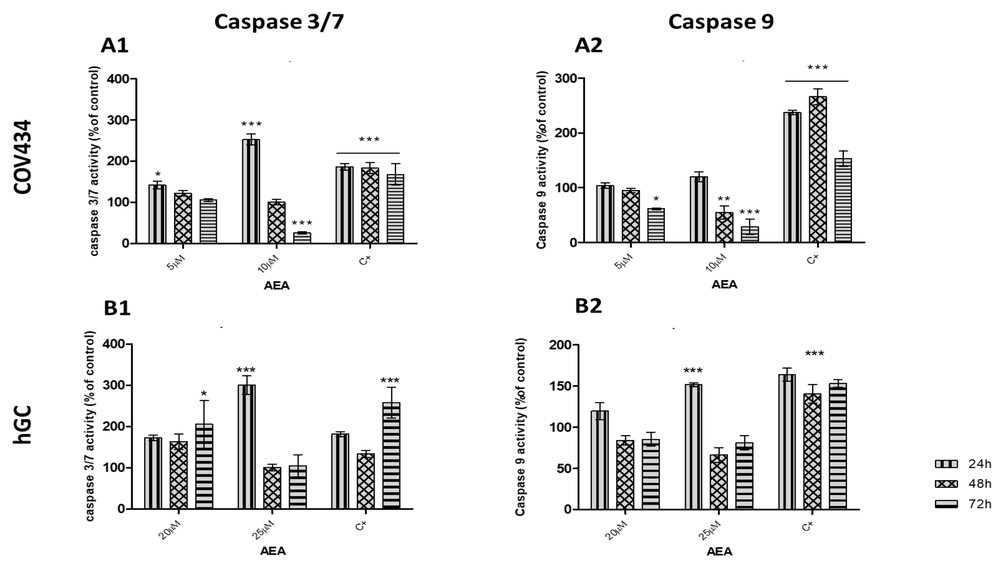


Figure 3 - COV434 and hGC morphological studies: Phase contrast and Höechst stain. COV434 and hGC cells morphology was analysed in the absence (control) or presence of AEA (10 µM for COV434 and 25 µM for hGC) after 48 (COV434) and 72 hours (hGC). Results are shown from single representative of three independent experiments.

Figure 4 - Effect of AEA on COV434 and hGC caspase 3/7 and 9 activity at 24,48 and 72 hours. Changes in caspase 3/7 and 9 activity induced by AEA at different concentrations (5/10 µM for COV434; 20/25 µM for hGC). Etoposide (20 µM) was used as a positive control. Results are expressed as mean ± SEM of at least three independent experiments performed in triplicate. Significant differences between control and treated cells are denoted as *(p<0.0005), **(p<0.0005) and *** (p <0.0005).



CONCLUSION

This study supports the idea that ECS balance is crucial for folliculogenesis and oocyte quality, also indicating that deregulated AEA levels may compromise female fertility.

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