EFFECT OF PACAP ON APOPTOTIC AND ANGIGENIC PATHWAYS IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS

Fabian E1, Mester L2, Szabo A2, Reglodi D1, Kiss P1, Szabadfi K3, Atlasz T4, Gabriel R3, Tamas A1, Kovacs K2
1Department of Anatomy, PTE-MTA “Lendulet” PACAP Research Team; 2Department of Biochemistry and Medical Chemistry; 3Department of Experimental Zoology and Neurobiology; 4Department of Sportbiology; University of Pecs, Pecs, Hungary

INTRODUCTION

In the retina, the integrity of the pigment epithelial cells is critical for the photoreceptor survival and vision. PACAP is known to exert retinoprotective effects, against several types of retinal injuries in vivo, including optic nerve transection, retinal ischemia, excitotoxic injuries, UV-A-induced lesion and diabetic retinopathy. We have shown earlier that PACAP activates antiapoptotic pathways and inhibits proapoptotic signaling in retinal lesions in vivo. Furthermore, we have also described that PACAP has antiapoptotic effects in human pigment epithelial cells (ARPE cells). The aim of the present study was to investigate the possible mechanisms of the protective effect of PACAP in ARPE cells oxidative stress by investigating different intracellular signaling pathways by Western blotting as well as apoptotic or angiogenic molecules by molecular arrays.

MATERIALS AND METHODS

Cells from the ARPE-19 cell line were cultured and exposed to 0.25 mM H2O2, for 3 hours to induce oxidative damage. PACAP-treated cells were exposed to 100 nM and 10 nM PACAP38.

Apoptosis and Angiogenesis Array Kit - apoptosis as well as angiogenesis arrays were performed from cell homogenates using Human Apoptosis and Angiogenesis Array kits from R&D Systems. Both arrays were performed as described by the manufacturer.

Western Blot - ARPE cells were treated with 250 mM H2O2 for 24 hours and 100 nM PACAP38. Samples were processed for Western blot analysis with the following primary antibodies: phospho-specific anti-Akt-1 Ser473 (1:1000; R&D Systems), phospho-specific anti-ERK1/2 Thr202/Tyr204, phospho-specific anti-SAPK/JNK Thr183/Tyr185, phospho-specific anti-p38 MAPK (1:1000; Cell Signaling Technology) anti-t-Akt (1:1000; Cell Signaling Technology) and anti-actin (1:5000; Sigma-Aldrich). Membranes were added horseradish peroxidase-conjugated secondary antibody (1:3000; BioRad). The antibody-antigen complexes were visualized by means of enhanced chemiluminescence.

RESULTS

Western Blot analysis - H2O2 treatment caused a strong activation of the proapoptotic p38MAPK and JNK pathways and PACAP treatment could decrease this activation. PACAP alone caused activation of the protective ERK and Akt pathways which was further activated after oxidative stress (Fig. 1).

Apoptosis array - oxidative stress increased the activation of bad, bax, HIF-1α, several heat shock proteins, while PACAP treatment decreased them (Fig. 2).

Angiogenesis array - oxidative stress induced the activation of proangiogenic factors, while PACAP treatment could decrease most of them (Fig. 3).

CONCLUSION

Our results show that PACAP influences numerous apoptotic markers in direction of an overall antiapoptotic effect and also regulates angiogenetic processes in pigment epithelial cells.

Figure 1. Western blot analysis of the different pro- and antiapoptotic factors in each group shows the ameliorative effects of PACAP in H2O2-damaged ARPE cells. Akt and ERK activation was markedly induced by PACAP treatment. In contrast, the strong activation of p38 and JNK was decreased by PACAP treatment in cells exposed to oxidative stress.

Figure 2. Apoptosis array to detect the protective effects of different doses of PACAP.

The main markers altered by PACAP treatments are framed.

Figure 3. Angiogenesis array to compare the effects of different doses PACAP treatment in ARPE cells exposed to H2O2 toxicity.

Supported by OTKA K72592, CNK78480, ETT278-04/2009, Bolinyi Scholarship, Richter Foundation, MTA “Lendulet” Program, SROP-4.2.2-B-10/1-2010-0029