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INTRACELLULAR NO AND CALCIUM IONS CHANGE CAUSED BY EXENDIN-4 IN THE PIG ILIAC ENDOTHELIUM

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Objectives To investigate the effects of Exendin-4 (Ex-4) on nitric oxide (NO) produced by pig iliac endothelium cells (PIEC) and the relationship with intracellular calcium ions change.

Methods GLP-1 receptor (GLP-1R) in the PIEC membrane was detected by immunofluorescence. PIEC were cultured in RPMI 1640 (with calcium)/DMEM (non calcium) nutrient medium separately and were treated with various concentrations of Ex-4. NO production and the intracellular calcium concentration were measured using Griess reaction in cell culture supernatants and Flow-Cytometer with the fluorescence probe Fluo-2 AM, respectively. The relationship between EX-4 and NO release was evaluated by measuring NO production before and after pretreated by eNOS blocker L-NAME.

Results GLP-1R was expressed in the PIEC. PIEC cultured in DMEM or RPMI 1640 were incubated with six different concentrations of Ex-4 (0.5, 1, 2, 4, 8 $\mu\text{g/ml}$). The NO productions of PIEC in DMEM were 1.39 ± 0.06 , 1.38 ± 0.01 , 1.33 ± 0.00 , 1.61 ± 0.01 and 2.42 ± 0.08 $\mu\text{mol/l}$, and the productions of cells in RPMI 1640 were 1.41 ± 0.08 , 1.57 ± 0.13 , 1.46 ± 0.09 , 1.54 ± 0.09 and 4.07 ± 0.17 . In different culture medium, the NO production of cells processing with 8 $\mu\text{g/ml}$ Ex-4 were significantly higher than all the other concentrations ($p < 0.05$). PIEC were then treated with 8 $\mu\text{g/ml}$ Ex-4 at a series of time points from 2 h, 4 h, 8 h, 12 h to 24 h. The NO productions increased gradually (0.53 ± 0.02 , 0.61 ± 0.04 , 1.44 ± 0.07 , 1.02 ± 0.06 , 0.13 ± 0.06) and reached the peak 8 h later ($p < 0.05$ vs 0.53 ± 0.02), and then slowly fell to the normal. Together with a higher intracellular calcium concentration when compared with the control group (57.46 ± 1.56 vs 62.38 ± 1.84 , $p < 0.05$).

No production in two L-NAME processing groups (L-NAME 10 $\mu\text{mol/l}$ and 100 $\mu\text{mol/l}$) were 2.21 ± 0.02 and 2.08 ± 0.31 , which were significantly decreased when compared to the non L-NAME pretreated group (2.32 ± 0.16 , $p < 0.05$). The NO production of both L-NAME groups was not significant ($p > 0.05$).

Conclusions GLP-1R was expressed in the PIEC. Ex-4 increased NO production in the endothelium cell through activation of eNOS in a dose and time dependent manner, and calcium may play an important role in the process.