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论著

Ghrelin对血管紧张素Ⅱ诱导的脐静脉内皮细胞氧化应激和内皮功能损伤的影响

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摘要：

目的：探讨促生长激素释放肽（ghrelin）对血管紧张素Ⅱ（angiotensinⅡ, Ang Ⅱ）诱导的离体培养的脐静脉内皮细胞（human umbilicus vein endothelial cell-12，HUVEC-12）损伤的保护作用。方法：(1)在培养的人脐静脉内皮细胞中加入10-9~10-6mol/L AngⅡ共培养24 h，或用10-9~10-6mol/L ghrelin预处理2 h后与10-6mol/L AngⅡ共培养24 h。用MTT法测量内皮细胞活力和用AnnexinV-FITC凋亡试剂盒在流式细胞仪下测量内皮细胞凋亡率。(2)HUVEC用10-9，10-8，10-7，10-6mol/L AngⅡ分别培养3，6，12或24 h，10-9，10-8，10-7或10-6 mol/L ghrelin预处理2 h后与10-6mol/L AngⅡ共培养24 h。生长激素促分泌剂受体1a (growth hormone secretagogue receptor 1a,GHSR1a)受体阻断剂 ［D-Lys3］GHRP-6加入 10-6 mol/L ghrelin预处理2 h后与10-6mol/L AngⅡ共培养24 h组，DCF荧光探针法测细胞内活性氧（reactive oxygen species, ROS）。(3)HUVEC分别与 10-9，10-8，10-7或10-6mol/L AngⅡ和 ghrelin共培养24 h，与10-6mol/L AngⅡ孵育3，6，12或24 h，或10-9，10-8，10-7或10-6mol/L ghrelin预处理30 min，1 h或2 h后与10-6mol/LAngⅡ培养24 h，加入丝裂原活化蛋白激酶/细胞外信号调节激酶信号通路（mitogen-activated protein kinase /extracullar signal regulated kinase 1/2，MAPK/ERK1/2）信号通路抑制剂PD98058、磷脂酰肌醇3-激酶/丝-苏氨酸激酶（phosphoinositide-3-kinase/serine threonine kinase，PI3K/Akt ）阻断剂 wortmannin和［D-Lys3］GHRP-6 共培养24 h，与用AngⅡ和ghrelin孵育的HUVEC 比较上清液中NO产量，HUVEC用ghrelin，PD98059，wortmannin， ［D-Lys3］GHRP-6预处理2 h后与10-6mol/L AngⅡ共培养24 h，或用ghrelin加上PD98059，wortmannin及［D-Lys3］GHRP-6预处理2 h后与10-6mol/L AngⅡ共培养24 h。内皮细胞上清中的一氧化氮（nitric oxide, NO）用Griess法测量。(4)HUVEC用空白对照或AngⅡ在有或没有用ghrelin或ghrelin和wortmannin 一起预处理的情况下孵育，用免疫印迹法（Western blot ）测量内皮型一氧化氮合酶（endothelial nitric oxide synthase, eNOS）的蛋白表达及丝苏氨酸激酶（serine threonine kinase，Akt）磷酸化蛋白表达。结果：AngⅡ引起内皮细胞损伤，增加HUVEC细胞凋亡率，减少培养的HUVEC细胞上清中NO含量，而ghrelin保护HUVEC免受AngⅡ损伤；Ghrelin减少与AngⅡ共同孵育的HUVEC ROS的产生。这种作用被［D-Lys3］GHRP-6消除。PD98059能阻止AngⅡ导致的HUVEC分泌NO减少，Wortmannin和［D-Lys3］GHRP-6消除Ghrelin保护HUVEC释放NO的作用；AngⅡ减少eNOS 的表达，但ghrelin能增加eNOS表达，Wortmannin消除Ghrelin的这种作用；Ghrelin 能刺激p-Akt的表达并在10~20 min达到高峰。结论：Ghrelin在AngⅡ导致的HUVEC损伤中起保护作用，其机制与通过GHSR1a受体减少氧化应激、增加eNOS蛋白表达和激活PI3K/Akt信号通路有关。

关键词： 促生长激素释放肽 血管紧张素Ⅱ 活性氧 一氧化氮 磷脂酰肌醇3-激酶/丝-苏氨酸激酶 内皮型一氧化氮合酶

Effect of ghrelin on angiotensin Ⅱ induced human umbilicus vein endothelial cell oxidative stress and endothelial cell injury

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Abstract:

ObjectiveTo determine the effect of ghrelin on protecting the human umbilical vein endothelial cells (HUVEC) from injury by angiotesin Ⅱ(AngⅡ) in vitro. Methods(1)HUVEC was incubated for 24 h with AngⅡ whose final concentration in the medium varied from 10-9 to 10-6mol/L or pretreated with 10-9to 10-6mol/L ghrelin for 2 h before incubation for 24 h with AngⅡwhose final concentration in the medium was 10-6mol/L. HUVECs were harvested to measure the cell vitality and cell apoptosis. The cell vitality was determined by MTT and cell apoptosis rates were measured by Annexin V-FITC apoptosis detection kit. (2)HUVECs were incubated for 3，6，12，or 24 h with 10-9，10-8，10-7，or 10-6mol/L AngⅡ, respectively. Before HUVECs were incubated with 10-6 mol/L AngⅡfor 24 h, ghrelin (10-9，10-8，10-7， and 10-6 mol/L) was used to pretreat the cells for 2 h. Growth hormone secregogue receptor 1a blocker ［D-Lys3］GHRP-6 was added to the cells which were incubated for 24 h with 10-6mol/L AngⅡ and pretreated with 10-6 mol/L ghrelin for 2 h. Cell reactive oxygen species were measured by dichlorofluorescin (DCF) fluorescence probe method. (3)HUVECs were incubated for 24 h with 10-9，10-8，10-7， or 10-6mol/L AngⅡ and ghrelin, respectively，and then were incubated with 10-6mol/L of AngⅡ for 3，6，12，or 24 h. Furthermore，HUVECs were pretreated with 10-9，10-8，10-7， or 10-6mol/L ghrelin for 30 min，1 h，or 2 h, and then were incubated with the inhibitor of mitogen-activated protein kinase /extracellular regulated kinase (MAPK/ERK1/2),PD98059， the inhibitor of phosphoinositide-3-kinase/serine threonine kinase(PI3K/Akt)wortmannin, and ［D-Lys3］GHRP-6 for 24 h. NO production was compared among groups. HUVECs were pretreated with ghrelin，PD98059， wortmannin， and ［D-Lys3］GHRP-6 for 2 h and co-cultured with 10-6mol/L AngⅡfor 24 h，or pretreated with ghrelin plus PD98059, wortmannin， and ［D-Lys3］GHRP-6 before incubation with AngⅡfor 24 h. NO was measured in the endothelial cell supernatants by Griess method. (4)HUVECs were cultivated with blank or AngⅡwith or without pretreatment with ghrelin or both ghrelin and wortmannin. The protein expression of eNOS and phospho-protein expression of Akt were measured by Western blot analysis. ResultsAngⅡinjuried the endothelial cell vitality，increased the cell apoptosis rates in HUVECs， and decreased NO production in HUVEC supernatants，whereas ghrelin protected HUVECs from AngⅡ injury. Ghrelin decreased the reactive oxygen species in HUVECs induced by AngⅡ. The effect could be attenuated by ［D-Lys3］GHRP-6 pretreatment； PD98059 alleviated AngⅡ inhibition of NO production in HUVEC supernatants. Wortmannin and ［D-Lys3］GHRP-6 could abolish protection of ghrelin from reducing NO production in

HUVEC supernatants. AngⅡreduced the expression of eNOS，but ghrelin increased eNOS expression. Wortmannin could cancel this effect of ghrelin. Ghrelin increased p-Akt expression and reached the peak in 10 and 20 min. ConclusionGhrelin may protect HUVECs from AngⅡinduced injury， which is related to decreasing oxidative stress， increasing the protein expression of eNOS， and activating PI3K/Akt signal pathway through GHSR1a receptor.

Keywords: ghrelin；angiotensin Ⅱ；reactive oxygen species；nitric oxide；phosphoinosi-tide-3-kinase/serine threonine kinase；endothelial nitric oxide synthase

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