Propofol於牛主動脈內皮細胞模式中對粒線體功能與細胞內鈣移動之影響

Effects of propofol on mitochondrial function and intracellular calcium shift in bovine aortic endothelial model

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摘要

背景：臨床上使用 propofol 時，常會引起血壓下降的現象，其機轉推論可能與內皮細胞內鱸濃度變化導致血管張力改變有關。本實驗以牛主動脈內皮細胞模式，探討 propofol 對於內皮細胞粒線體膜電位與形態造成之影響，以推論其與細胞內鈣離子移動及臨床現象之可能相關性。方法：本研究以培養之牛主動脈內皮細胞株(Gm 7372a)爲主題，先以螢光染劑 Fluo-3 處理後，再與 0.01 mM 之 propofol 於 37℃下共存 30 分鍾，而後加入 ionomycin，於螢光色層光譜儀（共軛焦點顯微鏡）下，觀察鈣離子自細胞器質（粒線體等）釋放形成明亮「熱點」之情形，與對照組比較；並以各個細胞內十個不同區域、不同時間之螢光變化，測量 propofol 影響細胞內鈣移動之程度。另以 DiOc6 測量細胞粒線體膜電位在 propofol 存在下之影響；並以 FCCP 處理爲對照組，觀察 propofol 在 TM Ros 染色下對內皮細胞粒線體形態造成之影響，用以評估 propofol 對內皮細胞細胞內鈣離子移動之效應，是否與其對粒線體膜電位與形態之影響有關。結果：加入 ionomycin 之後，於 10-30 妙內即可於細胞質與核內產生極強烈之「熱點」，顯激離鈣離子自細胞器質（主要是粒線體）釋放出來，並於 3-5 分鍾內逐漸減弱其強度。如先以 0.01 mM Propofol 處理後，ionomycin 所能產生之鈣離子螢光亮點，於強度及持續時間上，與對照組比較，明顯受到抑制。以 DiOC6 觀察內皮細胞粒線體膜電位時發現，在 propofol 存在下，螢光強度明顯下降，顯示粒線體膜電位受到抑制；再者，以 TM Ros 對粒線體外型形態進行螢光染色時，如先以 propofol 處理細胞，粒線體外型邊界會呈斷裂或乳繆狀，顯示 propofol 對於內皮細胞粒線體膜電位及形態，會產生抑制或破壞之現象。結論：本實驗顯示內皮細胞在臨床濃度之 propofol 存在下，可以明顯地抑制因 ionomycin 所造成之細胞內鈣移動，並經由

對於內皮細胞粒一體膜電位之抑制，與對粒線體形態變化之影響，推論 propofol 造成之血管舒張，可能與其抑制內皮細胞內粒線體功能有關。

Abstract

Background: Hypotension was commonly encountered in clinical practice during induction of anesthesia with propofoL The purpose of this study is to investigate the effect of propofol on mitochondrial membrane potential and morphology so as to infer its relation with intracellular calcium mobilization in bovine aortic endothelium. Methods: In this study, we used the cultured bovine aortic endothelial cells (Gm 7372a) to elucidate the impact of propofol upon the membrane potential and morphology of mitochondria in correlation with its effect on intracellular calcium shift. The intracellular calcium mobilization within the cells preincubated with or without propofol was evaluated using a fluorescent spectrophotometer (confocal microscope) after being treated with Fluo-3. The mobilization of intracellular calcium was demonstrated by the appearance of "hot spots" released from intracellular stores after the addition of an ionophore, ionomycin, to the incubation system. The membrane potential of mitochondria was measured by DiO C6 and the morphology of the mitoc hondria was evaluated by the treatment of TM Ros and compared with that by the treatment of the uncoupler, FCCP, as control. Results: The release ofcalcium "hot spots" from the intracellular stores (eg. mitochondria) after the addition of ionomycin was visualized to decrease dramatically within the endothelial cells after preincubation with propofol. The membrane potential of mitochondria was significantly inhibited by pretreatment of propofol at 0.01 mM, 37℃ for 30 min. Morphologically, the integrity of mitochondria was distorted and fragmented in the presence of propofol as compared with that of control. Conclusions: Our data showed that propofol in clinical concentration, 0.01 mM, could inhibit intracellular calcium shift from the intracellular stores and decrease the membrane potential and distort the morphology of mitochondria in bovine aortic endothelial cells. These inhibitions of the function and disfiguration of the morphology of mitochondria signify that the clinical hypotension induced by propofol might be of a potential mechanism.