Annexin 2 in Remifentanil-Induced Hyperalgesia

Authors: Xiaojie Cheng[1], Tony Gin[1], William KK Wu[1], Matthew TV Chan[1]

[1]Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong

Introduction: Emerging evidence suggests that -opioid receptor interacting protein (MORIP) contribute to the development of remifentanil-induced hyperalgesia. This study aimed to evaluate the role of a novel MORIP – Annexin 2 (ANXA2) in MOR membrane trafficking and to determine whether MORIP knockdown affect the development of remifentanil-induced hyperalgesia in a rat model.

Methods: In a series of cell experiments, we identified MOR signaling complexes using formaldehyde cross-linking immunoprecipitation and high-performance liquid chromatography-mass spectrometer/mass spectrometer. Following extensive bioinformatics analysis, functional annotation and literature reviews, ANXA2 was selected for further study. MOR and ANXA2 interaction was determined using immunoprecipitation. Confocal microscopy was used to reveal the effect of remifentanil and ANXA2 interaction on MOR membrane trafficking. In animal experiments, small interference RNA (siRNA) was injected intrathecally to rats for knockdown of ANXA2 expression. Thermal hyperalgesia was measured. Rats were then euthanized and their lumbar spinal cord and dorsal root ganglia (DRG) were collected for determination of MOR and ANXA2 expression.

Results: ANXA2 co-localized with MOR in the neurons of lumbar spinal cord (Fig A). We further visualized internalization of MOR after 15-minute exposure to remifentanil and that overexpression of ANXA2 facilitated remifentanil-induced internalization of MOR (Fig B). We also observed that the expression of ANXA2 was up-regulated at 24 hours with remifentanil treatment and this was accompanied by a decrease in MOR expression (Fig C). In a rat model of remifentanil-induced hyperalgesia, intrathecal injection of specific siRNA decreased ANXA2 protein level in DRG and was associated with an increase in thermal hyperalgesia (Fig D).

Conclusion: ANXA2 was as a novel MORIP that contributed to remifentanil-induced hyperalgesia. In a rat model, the knockdown of ANXA2 exacerbated hyperalgesia with remifentanil infusion.

Figure A: Validation of MOR-ANXA2 interaction using co-immunoprecipitation (IP) and reverse co-IP in rat lumbar spinal cords. **B:** Effect of ANXA2 on the MOR trafficking. Confocal microscopy of MOR trafficking, with quantitative analysis of red puncta of cells, puncta number was counted and error bars are standard deviation, $^{**}p<0.01$ (unpaired *t*-test). **C:** Western blot and quantitative analysis showing effects on ANXA2 and MOR protein expression with remifentanil-treatment, $^{*}p<0.05$ (unpaired *t*-test). **D:** Thermal hyperalgesia of the rats receiving intrathecal injection of siRNA and tail vein injection of remifentanil.