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Gas-pressure induced calcium release is oxygen dependent in HeLa cell layers
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Background: Calcium signaling in cells controls a number of diverse pathways associated including apoptosis. We asked whether calcium responses are involved in effect induced by volatile anesthetics and pressure to epithelial cells. **Methods:** A gas tight chamber was constructed with a glass coverslip sealed to the bottom allowing microscopical observation during the experiment. Nitrogen, air and volatile anesthetics were applied to a semiconfluent cell layer of HeLa cells which served as a model for epithelial cells. All gases were humidified and warmed to 32°C and tested as a static pressure of 1 bar. Following labeling with the calcium dye, Fluo-4, the calcium responses were followed by confocal microscopy using a Leica TCS-NT (excitation 488nm and emission > 515nm, argon/krypton laser). About 20 cells could be monitored simultaneously. At the end of the experiment, the cell layer was stained with 250µg/ml of propidium iodide (PI) to quantify apoptotic cells. **Results:** All oxygen-containing gases as well as the volatile anesthetic N₂O (21%O₂, 100%O₂ and 70%N₂O) gave rise to intracellular Ca²⁺ in HeLa cells as early as 1min after application of a constant pressure of 1 bar (160mmHg). No such response was observed in pure nitrogen and also not in pure xenon gas (100%N₂ and 100%xenon). The Ca²⁺ spikes declined after 5min and rapidly returned to baseline even when pressure persisted for the rest of the observation time (120min). Gadolinium chloride (Gd, 40mM) or EGTA (50mM) entirely abolished the [Ca²⁺]_i rise after the 3rd min and considerably depressed intracellular Ca²⁺ amplitude at the 1st min. The combination of both Bapta (100µM) with EGTA or Gd fully inhibited calcium oscillations. A number of other inhibitors applied (Ryanodine, paxilline: 1µM, Xestospongine C: 10µM, oxATP: 1µM) clearly demonstrated that the oxygen dependent, pressure induced calcium waves involved both, intracellular calcium stores and extracellular calcium as well. The gas pressure stimulated PLC activation to generate IP₃-induced intracellular Ca²⁺ release which in turn most likely opened store operated calcium channels (SOC), and possibly also calcium release activated channels (CRAC) in epithelial cells. The blockade by paxilline further suggests a role by large conductance calcium-activated K⁺ channels (BK). Upon decline of the calcium response, strong fluorescent labeling was transiently detected in the mitochondria. In some experiments, we observed massive swelling of the mitochondria and/or blebbing of the whole cells. This results of rapid apoptosis occurring within less than 30 min of the experiment was restricted to those where we applied gases with a high oxygen content (O₂>N₂O>air). Careful examination of the subcellular compartment in Fluo4-labelled cell layers demonstrated that the capacity of mitochondria to take up cytoplasmic calcium was the limiting factor leading to apoptosis. No such apoptosis was ever observed when oxygen free gases were applied. **Conclusion:** Gas-pressure induced intracellular calcium signaling does not cause apoptosis by itself but mitochondrial overload with calcium may eventually lead to cell death in epithelial cells. This apoptosis is calcium dependent and calcium waves are dependent on oxygen contents in gases applied.