Title: ANESTHETIC-INDUCED MODULATION OF PHOSPHOLIPID HYDROLYSIS BY SURFACE PRESSURE IN A MONOLAYER MODEL

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General anesthesia occurs through the reversible attenuation of central nervous system function, that is, synaptic transmission. A compression-induced increase in phospholipid (PL) monolayer surface pressure (SP, dynes/cm) above 35 dynes/cm abolishes monolayer PL hydrolysis by phospholipase-C (PLC).[1] It is noteworthy that general anesthetics at clinical concentrations increase SP of PL monolayers.[2] Because PL hydrolysis is central to mechanisms of membrane transduction, we hypothesized that anesthetic-induced increase in membrane SP inhibits synaptic transmission by inhibiting PL hydrolysis and membrane transduction. Our objective was to determine the relationship between anesthetic-induced increase in PL monolayer SP and the rate of PL hydrolysis by PLC. A Wilhelmy plate and microbalance measured SP of a dipalmitoylphosphatidyl-^{14}C-choline (DPPC) monolayer on Krebs Ringer bicarbonate buffer (pH 7.3) at 37°C. A Geiger-Mueller tube/ratemeter monitored monolayer ^{14}C activity. Halothane (H) 0-4% in air was vaporized over the DPPC monolayer. Initial SP before hydrolysis with Bacillus cereus PLC was varied between 3 and 31 dynes/cm. The rate of DPPC hydrolysis (i.e., decrease in monolayer ^{14}C activity with time, counts per min (cpm)/min) had an initial lag phase whose duration varied directly with SP, and a rapid hydrolysis phase, whose rate increased with SP until cut-off. With a constant amount of DPPC and PLC, increasing amounts of H through increased SP prolonged the duration of the lag phase and increased the rapidity of hydrolysis. The slope of the rapid hydrolysis segment was then related to the initial SP at time of addition of PLC (Figure 1). At SP between 3 and 24 dynes/cm, the rate of hydrolysis was unaffected by SP and constant at 350 cpm/min. It abruptly increased fivefold to 1600 cpm/min at SP between 24 and 30 dynes/cm. PL hydrolysis ceased abruptly (i.e., infinitely long lag phase) at SP greater than 30 dynes/cm. These results suggest that H affects DPPC hydrolysis by PLC through membrane SP rather than by directly acting on the enzyme. The implications of our findings also relate to the regulation of membrane composition and function. They suggest that the composition and function of membranes could be controlled by the differential activation and suppression of various enzymes subject to membrane SP each operating within its own narrow range of SP as observed for PLC. The fivefold activation of PLC occurs with a SP change of only 6 dynes/cm, which agrees with similar observations for acetylcholinesterase.[3] The significance of these data is underscored by the finding that addition of a single double bond to C 16:0/C18:0 PC could increase SP by as much as 20 dynes/cm. The demonstrated high sensitivity of PLC activation to membrane SP and in turn, the sensitivity of membrane SP to membrane lipid composition has broad implications for the effects of anesthetics on the physicochemical determinants of enzyme activation.

References

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