

# Calcium and Calmodulin Regulate Mercury-induced Phospholipase D Activation in Vascular Endothelial Cells

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Earlier, we reported that mercury, the environmental risk factor for cardiovascular diseases, activates vascular endothelial cell (EC) phospholipase D (PLD). Here, we report the novel and significant finding that calcium and calmodulin regulated mercury-induced PLD activation in bovine pulmonary artery ECs (BPAECs). Mercury (mercury chloride, 25  $\mu$ M; thimerosal, 25  $\mu$ M; methylmercury, 10  $\mu$ M) significantly activated PLD in BPAECs. Calcium chelating agents and calcium depletion of the medium completely attenuated the mercury-induced PLD activation in ECs. Calmodulin inhibitors significantly attenuated mercury-induced PLD activation in BPAECs. Despite the absence of L-type calcium channels in ECs,

nifedipine, nimodipine, and diltiazem significantly attenuated mercury-induced PLD activation and cytotoxicity in BPAECs. This study demonstrated the importance of calcium and calmodulin in the regulation of mercury-induced PLD activation and the protective action of L-type calcium channel blockers against mercury cytotoxicity in vascular ECs, suggesting mechanisms of mercury vasculotoxicity and mercury-induced cardiovascular diseases.

**Keywords:** calcium; calmodulin; L-type calcium channel blockers; lipid signaling; mercury; phospholipase D; phosphatidic acid; vascular endothelial cells

Mercury (Hg), a heavy metal belonging to the transition metal series of the periodic table, is an established environmental pollutant with known toxicity in humans. Mercury is widely recognized for its cytotoxicity, neurotoxicity, and immunotoxicity, and it appears to play no known

physiological role.<sup>1-3</sup> Mercury usage in several devices causes accidental and occupational exposure to the metal among humans.<sup>1</sup> Inorganic mercury, in the form of chloride, is toxic to many organisms, including humans, and can readily undergo microbial biomethylation to form the highly toxic organic form, methylmercury.<sup>4</sup> Methylmercury has been shown to cause hypertension in rats.<sup>5</sup> It has also been documented that methylmercury generates reactive oxygen species (ROS), leading to cellular oxidative stress.<sup>6,7</sup> Elemental mercury (Hg<sup>0</sup>) was commonly used in dental practices for the greater part of the 20th century, and mercury vapor released from amalgam surfaces in the mouth is the predominant source of mercury exposure in the general population.<sup>8</sup> Persistent use of thimerosal (an organic mercurial)

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