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THE SULFHYDRYL REAGENT THIMEROSAL ELICITS HUMAN PLATELET AGGREGATION BY MOBILIZATION OF INTRACELLULAR CALCIUM AND SECONDARY PROSTAGLANDIN ENDOPEROXIDE FORMATION

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Summary. The effect of the sulfhydryl (SH) group inhibitor ethylmercurithiosalicylate (thimerosal) on the function of human platelets was investigated. In contrast to known SH reagents such as *p*-chloromercuribenzoate or N-ethylmaleimide, thimerosal elicited both aggregation and [^{3}H]serotonin release of washed human platelets at low micromolar concentrations ($\geq$ 2 μM). Only a significant higher dose ($\geq$ 15 μM) was effective when platelets were pretreated with the cyclooxygenase inhibitor aspirin, indicating an amplification of the proaggregatory effect of thimerosal by secondary prostaglandin (PG) endoperoxide and/or thromboxane (TX) formation. Consistent with this notion, thimerosal induced endogenous platelet arachidonic acid (20:4) metabolism which could be attributed to enhanced 20:4 liberation, presumably by activation of phospholipase A_2. The latter effect was mediated by mobilization of intracellular calcium (Ca^{2+}), and was not affected by removal of extracellular Ca^{2+}. In the presence of aspirin, the thimerosal-induced Ca^{2+} elevation was completely reversed by dithiothreitol (DTT) which implicates SH groups in intracellular Ca^{2+} transport. In contrast to previous observations with other SH reagents, thimerosal had no effect on the inositoltrisphosphate (IP_3)-mediated release or the sequestration (and/or extrusion) of intracellular Ca^{2+} following stimulation with thrombin, indicating an action on an as yet undefined Ca^{2+} transport system.

Platelet membranes contain SH groups essential for the maintenance of platelet integrity and function, and SH reagents affect platelet function by binding to SH and disulfide groups of platelet membranes [1]. The organic mercury compound thimerosal elicits aggregation of platelet-rich plasma and serotonin release, presumably by such a mechanism [2,3]. Moreover, thimerosal induces release of the endothelium-derived relaxing factor (EDRF) from endothelial cells, probably a Ca^{2+}-mediated process [4,5], and stimulates arachidonic acid (20:4) metabolism in human platelets and murine peritoneal macrophages [6]. The latter effect has been attributed to inhibition of 20:4 reacylation leading to an increased level of free 20:4 within the cell [7], generally accepted to be the limiting factor in eicosanoid biosynthesis [8]. On the contrary, esterified 20:4 can be liberated from cellular (phospho)lipids by phospholipase A_2 in response to an elevation of the intracellular Ca^{2+} level by various agonists [9]. Platelets convert 20:4 mainly to the PG endoperoxide PGH_2 which is subsequently metabolized to 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and TXA_2 by TX synthase [10]. PGH_2 and in particular TXA_2 are powerfull platelet agonists which stimulate phosphatidylinositol (PI) metabolism [11]. The present study addresses the question by which mechanism thimerosal increases the level of free 20:4 in platelets and whether this effect accounts for its proaggregatory activity.

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